Progress in Neurobiology 85 (2008) 335–353

Contents lists available at ScienceDirect

Progress in Neurobiology

journal homepage: www.elsevier.com/locate/pneurobio

Physiological effects of melatonin: Role of melatonin receptors and signal transduction pathways

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ARTICLE INFO

Article history:
Received 27 August 2007
Received in revised form 22 January 2008
Accepted 9 April 2008

Keywords:
Melatonin
Melatonin receptors
Suprachiasmatic nuclei
Dopaminergic system
Blood vessels
Immune system
Prostate
Mammary glands
Alzheimer's disease
Parkinson's disease

ABSTRACT

Melatonin, an endogenous signal of darkness, is an important component of the body's internal time-keeping system. As such it regulates major physiological processes including the sleep wake cycle, pubertal development and seasonal adaptation. In addition to its relevant antioxidant activity, melatonin exerts many of its physiological actions by interacting with membrane MT_1 and MT_2 receptors and intracellular proteins such as quinone reductase 2, calmodulin, calreticulin and tubulin. Here we review the current knowledge about the properties and signaling of melatonin receptors as well as their potential role in health and some diseases. Melatonin MT_1 and MT_2 receptors are G protein coupled receptors which are expressed in various parts of the CNS (suprachiasmatic nuclei, hippocampus, cerebellar cortex, prefrontal cortex, basal ganglia, substantia nigra, ventral tegmental area, nucleus accumbens and retinal horizontal, amacrine and ganglion cells) and in peripheral organs (blood vessels, mammary gland, gastrointestinal tract, liver, kidney and bladder, ovary, testis, prostate, skin and the immune system). Melatonin receptors mediate a plethora of intracellular effects depending on the cellular milieu. These effects comprise changes in intracellular cyclic nucleotides (cAMP, cGMP) and calcium levels, activation of certain protein kinase C subtypes, intracellular localization of steroid hormone receptors and regulation of G protein signaling proteins. There are circadian variations in melatonin receptors and responses. Alterations in melatonin receptor expression as well as changes in endogenous melatonin production have been shown in circadian rhythm sleep disorders, Alzheimer's and Parkinson's diseases, glaucoma, depressive disorder, breast and prostate cancer, hepatoma and melanoma. This paper reviews the evidence concerning melatonin receptors and signal transduction pathways in various organs. It further considers their relevance to circadian physiology and pathogenesis of certain human diseases, with a focus on the brain, the cardiovascular and immune systems, and cancer.

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Abbreviations: AANAT, arylalkylamine-*N*-acetyl transferase; AD, Alzheimer's disease; AFMK, N^1-acetyl-N^2-formyl-5-methoxykynuramine; AMMTC, *N*-acetyl-4-amino-methyl-6-methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole; IL, interleukin; cAMP, 3′,5′-cyclic adenosine monophosphate; cGMP, 3′,5′-cyclic guanosine monophosphate; CREB, cyclic AMP responsive element binding; CRSD, circadian rhythm sleep disorders; DA, dopamine; DSPS, delayed sleep phase syndrome; ER, estrogen receptor; GABA, γ-aminobutyric acid; GnRH, gonadotropin-releasing hormone; GPR, G protein receptor; GTP, guanosine triphosphate; HIOMT, hydroxyindole-*O*-methyltransferase; LH, luteinizing hormone; NE, norepinephrine; NK, natural killer; PKC, protein kinase C; PT, pars tuberalis; RGCs, retinal ganglion cells; RGS, regulator of G protein signaling; RPE, retinal pigment epithelium; SCN, suprachiasmatic nucleus.

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doi:10.1016/j.pneurobio.2008.04.001

Contents

1. Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine) was first isolated and identified by Lerner et al. (1958). It is the major neurohormone secreted during the dark hours at night by the vertebrate pineal gland. Tryptophan serves as the precursor for melatonin biosynthesis, and is taken up from the circulation and then converted into serotonin. Serotonin is then converted into *N*-acetylserotonin by the enzyme arylalkylamine-*N*-acetyl transferase (AANAT) while *N*-acetylserotonin is metabolized into melatonin by the enzyme hydroxyindole-*O*-methyltransferase (HIOMT) (Axelrod and Wurtman, 1968). Once formed, melatonin is released into the capillaries and in higher concentrations into the cerebrospinal fluid (Tricoire et al., 2003) and is then rapidly distributed to most body tissues (Cardinali and Pevet, 1998).

Intravenously administered melatonin exhibits a biexponential decay with a first distribution half-life of 2 min and a second metabolic half-life of 20 min (Claustrat et al., 2005). Circulating melatonin is metabolized mainly in the liver where it is first hydroxylated by cytochrome P450 monooxygenases and then conjugated with sulfate to form 6-sulfatoxymelatonin (Skene et al., 2001). Melatonin is also metabolized by oxidative pyrrole-ring cleavage into kynuramine derivatives (Hirata et al., 1974). The primary cleavage product is N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), which is deformylated, either by arylamine formamidase or hemoperoxidases to N^1-acetyl-5-methoxykynuramine (Hardeland et al., 1993; Tan et al., 2007). Some evidence has suggested that pyrrole ring cleavage contributes to about one-third of the total melatonin catabolism, but the percentage may be even higher in certain tissues. It has been proposed that AFMK is the primitive and primary active metabolite of melatonin (Tan et al., 2007).

The circadian pattern of pineal melatonin secretion is regulated by the biological clock that resides in mammals within the hypothalamic suprachiasmatic nucleus (SCN) of the hypothalamus. Lesions in the SCN abolish the rhythm of pineal melatonin production in mammals (Klein and Moore, 1979). The SCN is synchronized to the environmental light–dark cycle by light perceived by the retina, acting mainly on a subgroup of retinal ganglion cells (RGCs) that contain the photopigment melanopsin (Berson et al., 2002). These RGCs connect to the SCN via the retinohypothalamic tract.

The SCN regulates pineal gland's function through a polysynaptic network involving the paraventricular nucleus of the hypothalamus. Descending polysynaptic fibers from these regions project through the medial forebrain bundle and the reticular formation to the intermediolateral horns of the cervical segments of the spinal cord (Buijs et al., 1998). Postganglionic sympathetic fibers from the superior cervical ganglia reach the pineal gland and regulate melatonin biosynthesis through the presynaptic release of norepinephrine (NE). NE release occurs during the "night" portion of the circadian pacemaker cycle provided that this occurs in a dark environment.

Activation of the pineal β-adrenergic receptors by NE results in increased 3′,5′-cyclic adenosine monophosphate (cAMP) concentration that promotes the biosynthesis of melatonin (Klein et al., 1971). α_1-Adrenergic receptors potentiate β-adrenergic activity by producing a sharp increase in intracellular Ca^{2+} and activation of protein kinase C (PKC) and of prostaglandin synthesis (Vacas et al., 1980; Ho and Klein, 1987; Krause and Dubocovich, 1990). The subcellular mechanisms involved in increase and turnoff of AANAT activity have been elucidated in great detail [see for references Maronde and Stehle, 2007]. Cyclic AMP stimulates AANAT expression and phosphorylation via protein kinase A, which also allows AANAT to be stabilized by binding of 14-3-3 proteins (Schomerus and Korf, 2005; Ganguly et al., 2005). The nocturnal exposure to bright light suppresses melatonin production immediately by degradation of pineal AANAT (Gastel et al., 1998).

It has now been demonstrated that melatonin is produced by many organs other than the pineal gland. These include the retina (Cardinali and Rosner, 1971a,b; Tosini and Menaker, 1998), gastrointestinal tract (Raikhlin and Kvetnoy, 1976; Bubenik, 2002), skin (Slominski et al., 2005), lymphocytes (Carrillo-Vico et al., 2004) and bone marrow (Conti et al., 2000).

Because pineal melatonin production occurs during the dark phase and is acutely suppressed by light, and, further, because melatonin is quickly cleared from the circulation following the cessation of its production, the time and duration of the melatonin peak reflect the environmental night period (Cardinali and Pevet, 1998). Plasma melatonin exhibits a circadian rhythm with high levels at night, and low levels during the day, attaining peak concentrations of plasma melatonin between 02:00 and 04:00 h. Longer nights are associated with a longer duration of melatonin secretion (Cardinali and Pevet, 1998). Hence melatonin is a signal of darkness that encodes time-of-day and length-of-day information to the brain including the SCN, brain and peripheral organs (Pandi-Perumal et al., 2006b). In mammals, melatonin is critical for the regulation of seasonal changes for various physiological,

neuroendocrine and reproductive functions (Reiter, 1980; Cardinali and Pevet, 1998). These actions of melatonin are processed in nuclei of the hypothalamus and in the pars tuberalis (PT) of the pituitary (Lincoln, 2006). These particular functions appear to be less relevant to humans and will therefore not be considered further in this review.

The other major function of melatonin is its regulation of the phase of circadian rhythms by a direct action on the SCN (Gillette and Tischkau, 1999). Melatonin administration has been shown to shift circadian rhythms in both rodents (Redman et al., 1983) and humans (Arendt and Skene, 2005; Cardinali et al., 2006). When administered once daily at the normal bedtime hour, melatonin (at doses ranging from 0.5 mg to 5 mg) entrains free running circadian rhythms of most blind subjects, and improves nocturnal sleep and daytime alertness (Arendt and Skene, 2005). The observations have been confirmed in other studies also (Lockley et al., 1995; Sack et al., 2000). In sighted subjects, melatonin has also been shown to maintain entrainment at a 5 mg dose (Middleton et al., 1997).

The timing of melatonin secretion is closely associated with the timing of sleep propensity and it also coincides with decreases in core body temperature, alertness and performance (Dijk and Cajochen, 1997; Rajaratnam and Arendt, 2001; Arendt, 2006). Melatonin has the capacity to alter the timing of mammalian circadian rhythms and functions in concert with light to synchronize circadian rhythms with the prevailing light–dark cycles. It has been used successfully in treating various circadian rhythm disorders such as delayed sleep phase syndrome (Dahlitz et al., 1991), shift-work sleep disorder (Folkard et al., 1993; Burgess et al., 2002; Srinivasan et al., 2006b), blindness or pathophysiological states of delayed/advanced sleep phase syndromes (Hagan and Oakley, 1995; Zisapel, 2001a; Arendt and Skene, 2005; Lewy et al., 2006; Cardinali et al., 2006).

Melatonin is reported to have a role in sleep initiation as the trigger for opening the circadian "sleep gate", acting as a sleep regulator (Shochat et al., 1997; Krauchi and Wirz-Justice, 2001; Zhdanova and Tucci, 2003; Pandi-Perumal et al., 2005; Dijk and von Schantz, 2005; Zisapel, 2007). Other actions of the hormone include inhibition of dopamine (DA) release in the hypothalamus and retina (Zisapel, 2001b), involvement in the aging process (Reiter et al., 1998; Karasek, 2004) and pubertal development (Waldhauser et al., 1988; Silman, 1991; Cavallo, 1992; Commentz et al., 1997; Salti et al., 2000), blood pressure control (Arangino et al., 1999; Cagnacci et al., 2001; Cavallo et al., 2004; Scheer et al., 2004; Grossman et al., 2006), free-radical scavenging (Tan et al., 2007) and regulation of the immune response (Srinivasan et al., 2005; Carrillo-Vico et al., 2006). If given during the day, when it is not present endogenously, melatonin has soporific effects which resemble its action at night, i.e., it lowers body temperature and induces fatigue while concomitantly producing a brain activation pattern resembling that which occurs during sleep (Gorfine et al., 2006, 2007; Gorfine and Zisapel, 2007).

Melatonin production decreases with age and in certain diseases, e.g., certain malignancies, Alzheimer's disease (AD) and cardiovascular disease (Brugger et al., 1995; Bartsch and Bartsch, 1999; Girotti et al., 2000, 2003; Zhou et al., 2003; Jonas et al., 2003; Pandi-Perumal et al., 2005). This decrease in melatonin output has been linked to insomnia in older patients (Haimov et al., 1994; Leger et al., 2004) and to a higher prevalence of cancer (Bartsch and Bartsch, 2006; Stevens, 2006; Schernhammer et al., 2006).

In this review, we will focus our attention on melatonin receptors and signal transduction pathways and their role in circadian physiology and pathogenesis of certain human diseases. For an in-depth discussion of other important functions of melatonin the reader is referred to recent reviews of its direct and indirect actions as an antioxidant (Hardeland, 2005; Reiter et al., 2007; Tan et al., 2007).

An important conceptual difficulty in melatonin research is that it is a signal of darkness, but has different functional consequences depending on the species' time of peak activity. In nocturnal species, melatonin is associated with arousal and physical activity whereas in diurnal species, it is associated with sleep and rest. Accordingly, administration of melatonin promotes sleep in humans (Dollins et al., 1994), but not in rats and mice (Huber et al., 1998; Mailliet et al., 2001). Because the SCN has a similar function in nocturnal and diurnally active animals, the differential "interpretation" of the melatonin signal must be downstream to the SCN, and possibly involves a counter-balance between melatonin's effects on brain regions that are involved in certain activities (e.g., arousal) and those involved in suppression of those activities. Similar considerations must be applied to different tissues and functions of melatonin in the body. Therefore, when applicable, the effects on human tissues and mechanisms are highlighted.

2. Melatonin receptors, their localization and regulation

Two mammalian subtypes of G protein coupled melatonin receptors, MT_1 (Mel 1a) and MT_2 (Mel 1b), have been cloned and characterized (Reppert et al., 1994, 1995; Dubocovich and Markowska, 2005). The human MT_2 receptor has a lower affinity (K_d = 160 pmol/l) for ^{125}I-melatonin as compared to the human MT_1 receptor (K_d = 20–40 pmol/l), but the binding characteristics of the two are generally similar, e.g., both are of high affinity and the agonist binding is guanosine triphosphate (GTP)-sensitive (Dubocovich and Markowska, 2005).

A third melatonin binding site initially called the "MT_3" was later characterized as the enzyme quinone reductase 2 (Nosjean et al., 2000). This enzyme belongs to a group of reductases that participate in the protection against oxidative stress by preventing electron transfer reactions of quinones.

In addition, functional and ligand binding studies have demonstrated the presence of low affinity (K_d in the 10 nm range) melatonin membrane binding sites in the preoptic area of the hypothalamus and in the medulla-pons in rats (Zisapel and Laudon, 1982; Laudon et al., 1988) and hamsters (Anis et al., 1989). While the density of these sites varies with the time of day and physiological conditions, compatible with the ability of melatonin to inhibit DA release in these areas (Zisapel, 2001b), the proteins involved in this activity have not been identified so far.

Melatonin is also a ligand for a retinoid related orphan nuclear hormone receptor (RZR/RORα) (Becker-Andre et al., 1994). These nuclear receptors belong to the RZR/ROR orphan receptor subfamily, which includes three subtypes (α, β, γ) and four splicing variants of the α-subtype ((Becker-Andre et al., 1993).

In addition, melatonin interacts with intracellular proteins such as calmodulin (Benitez-King and Anton-Tay, 1993), calreticulin (Macias et al., 2003) or tubulin (Cardinali and Freire, 1975; Melendez et al., 1996) and antagonizes the binding of Ca^{2+} to calmodulin (Benitez-King, 2006). These interactions are most likely related to some of the physiological effects of melatonin but critical data regarding this point have yet to be obtained.

MT_1 and MT_2 receptors are expressed both singly and together in various tissues of the body (Reppert et al., 1994,1995; Dubocovich and Markowska, 2005). Functional MT_1 receptors have been localized in the SCN (Liu et al., 1997; Dubocovich and Markowska, 2005), cerebellum (Al Ghoul et al., 1998), hippocampus (Savaskan et al., 2002a), central dopaminergic pathways (i.e., substantia nigra, ventral tegmental area, nucleus accumbens, caudate-putamen) (Uz et al., 2005), ovary (Clemens et al., 2001),

testis (Frungieri et al., 2005), mammary gland (Ram et al., 2002), retina (Scher et al., 2002), coronary blood vessels and aorta (Ekmekcioglu et al., 2001a,b), liver and kidney (Naji et al., 2004), gallbladder (Aust et al., 2004), skin (Slominski et al., 2003) and the immune system (Pozo et al., 2004). Melatonin MT_2 receptors are more restrictively expressed, being found mainly in the brain, although their presence has also been detected in the lung, cardiac, aortic and coronary tissue, myometrium and granulosa cells, immune cells, duodenum and adipocytes (Dubocovich and Markowska, 2005). The "MT_3 receptor" (i.e., quinone reductase 2) is expressed in the liver, kidney, brain, heart, lung, intestine, muscle and brown adipose tissue (Nosjean et al., 2001) and there is pharmacological evidence it is also present in the eye (Pintor et al., 2003).

Melatonin binding and receptor mRNA levels vary on a circadian basis, with expression levels affected by light and melatonin concentration in plasma. In the rodent SCN and PT, MT_1 mRNA expression and ^{125}I-melatonin binding exhibit daily variations, with elevated levels occurring during daytime. Light exposure during the night also increases ^{125}I-melatonin binding, coincident with the suppression of melatonin synthesis (Guerrero et al., 1999; Masson-Pevet et al., 2000), indicating that melatonin down-regulates some of its receptor population (Gilad et al., 1997a). Other studies involving manipulation of melatonin levels also support the view that the hormone regulates its own receptors (Recio et al., 1996; Masson-Pevet et al., 2000; Schuster et al., 2000, 2001).

In addition, there is rhythmic regulation of MT_1 mRNA expression and ^{125}I-melatonin binding that is independent of rhythmic melatonin secretion (Anis et al., 1989; Masson-Pevet et al., 2000; Schuster et al., 2000, 2001). Estradiol appears to regulate melatonin binding site density and affinity and responses in the rat hypothalamus (Zisapel et al., 1987; Laudon and Zisapel, 1987). Aging is associated with decreases ^{125}I-melatonin binding in the rat hypothalamus (Laudon et al., 1988; Oaknin-Bendahan et al., 1995). Indeed, aging and AD are associated with decreased MT_1 melatonin receptor expression in the human SCN and cortex (Brunner et al., 2006), although an increase in MT_1-receptor immunoreactivity in the hippocampus of AD patients has been reported (Savaskan et al., 2002a). The MT_2 melatonin receptor subtype is present in human retina, cortex and hippocampus and its expression level decreases in AD (Savaskan et al., 2005, 2007).

Collectively, these studies reveal the complexity of the mechanisms regulating melatonin receptor expression. It is therefore not surprising that melatonin-mediated responses are also dependent on circadian time, duration of exposure, presence of endogenous melatonin, and functional receptor sensitivity. This is discussed further below, with respect to individual organs or systems.

3. Melatonin's signal transduction mechanisms

The signal transduction pathways for melatonin receptors appear to vary among different tissues and cell types [for reviews, see von Gall et al., 2002; Witt-Enderby et al., 2003 and references cited therein]. By using recombinant melatonin receptors it has been shown that the MT_1 melatonin receptor is coupled to different G proteins that mediate adenylyl cyclase inhibition and phospholipase C beta activation. Thus MT_1 receptor activation leads to activation of a large variety of G proteins including $G_{i\alpha 2}$, $G_{i\alpha 3}$ and $G_{\alpha q}$ (Brydon et al., 1999), and $G_{\alpha s}$, $G_{\alpha z}$ and $G_{\alpha 16}$ (Ho et al., 2001; Chan et al., 2002). In some of these systems, melatonin has inhibitory effects on the cAMP signal transduction cascade (Niles and Hashemi, 1990; Brydon et al., 1999) including decreases in protein kinase A activity (Morgan et al., 1994) and nuclear factor CREB (cAMP responsive element binding protein) phosphorylation (McNulty et al., 1994). In the hypophyseal PT, the main effect of MT_1 receptor activation is the inhibition of cAMP accumulation (Morgan et al., 1994). However, in COS-7 cells (Chan et al., 2002) and human neuroblastoma SH-SY5Y cells (Schuster et al., 2005) transfected with cloned MT_1 receptors, melatonin causes stimulation of cAMP. Although the exact mechanism remains to be defined, this stimulatory effect was independent of an interaction with G_i or G_s proteins and was tentatively associated with a calcium-calmodulin signal transduction pathway and c-Jun N-terminal kinase activation (Chan et al., 2002; Schuster et al., 2005).

The MT_2 receptor couples to a number of signal transduction pathways including phosphoinositide production, the inhibition of adenylyl cyclase and the inhibition of soluble guanylyl cyclase pathway (Boutin et al., 2005). In NIH3T3 cells transfected with the human MT_2 melatonin receptors, high concentrations of melatonin were found to inhibit cAMP synthesis (Reppert et al., 1995; Jones et al., 2000). Additionally, in slices from the rat SCN the phase shifting effects of melatonin are apparently mediated by MT_2 receptors through the increase in PKC activity (McArthur et al., 1997; Hunt et al., 2001). On the other hand, in human benign and prostate cancer cells, melatonin has been associated with an increase in cyclic 3′,5′-cyclic guanosine monophosphate (cGMP) levels, presumably through its regulatory effects on G protein signaling (RGS), i.e., by redistributing and modulating G protein-mediated signaling (Gilad et al., 1997a, 1998a; Bubis and Zisapel, 1998, 1999; Rimler et al., 2007).

Melatonin inhibits stimulated DA release in the rat hypothalamus and rabbit retina (Zisapel and Laudon, 1982; Dubocovich, 1983; Zisapel, 2001b). This inhibition is associated with suppression of calcium uptake by the stimulated tissue (Zisapel and Laudon, 1983; Vacas et al., 1984). On the other hand, in human prostate cells the increase by melatonin of cGMP leads to enhanced calcium uptake through cyclic nucleotide gated channels (Rimler et al., 2007). Bidirectional effects of melatonin on calcium uptake in the hypothalamus have been documented at different times of the day (Rosenstein et al., 1991). Activation of MT_1 receptor also induces a transient elevation of cytosolic Ca^{2+} and inositol phosphate accumulation (Brydon et al., 1999; Roka et al., 1999).

The increase in cell calcium may lead to activation of PKC-α, as first demonstrated in human prostate epithelial cells (Gilad et al., 1997a; Sampson et al., 2006; Rimler et al., 2006). In these cells, in which melatonin brought about the nuclear exclusion of the androgen receptor via activation of PKC, melatonin induced membrane association of cytoplasmic PKC-α in a dose-dependent manner. PKC-α blocked melatonin effect on androgen receptors (Sampson et al., 2006). These data suggest that PKC-α activation is a critical step in melatonin action. This may also occur with MT_2 receptors (Godson and Reppert, 1997; Rivera-Bermudez et al., 2003; Gerdin et al., 2004b) and may be associated with down-regulation of melatonin receptors (Gerdin et al., 2004b). On the other hand, PKC activation by melatonin attenuates specific cellular functions such as androgen-dependent gene expression in prostate cells (Rimler et al., 2007) and mobilizes RGS proteins in the cells (Rimler et al., 2007). The PKC signaling pathway may thus be an important component of melatonin's circadian effects and may explain some of the multiplicity in G protein types that mediate melatonin's effects in cells.

MT_1 melatonin receptors can also regulate other ion fluxes and specific ion channels. Inhibition of the large conductance, calcium-activated potassium channel (BK_{Ca}) underlies vasoconstrictor effects due to MT_1 melatonin receptors (Geary et al., 1997, 1998). In this case, channel blockade may result from a decrease in the cAMP-protein kinase A pathway that phosphorylates BK_{Ca} channels. Inward rectifier potassium channels (Kir) are another

potential target for melatonin. MT_1 melatonin receptors expressed in Xenopus oocytes (Nelson et al., 1996) or AtT20 cells (Nelson et al., 2001) activate Kir3 (GIRK) inward rectifier potassium channels through a PTX-sensitive mechanism that may involve βγ subunits of G_i proteins. Activation of Kir3 channels may underlie melatonin-mediated increases in potassium conductance (Jiang et al., 1995) and inhibition of neuronal firing in the SCN [for references see Brown and Piggins, 2007]. Further discussion of melatonin signal transduction is given below.

4. Melatonin receptors in the suprachiasmatic nuclei

The physiological role of melatonin in the SCN has been studied in a number of animal models. Melatonin inhibits SCN multiunit activity by acting through MT_1 receptors (Liu et al., 1997). This effect is more pronounced during daytime when SCN neuronal activity is high, although it is also observed at night. The acute inhibitory effect of melatonin on SCN multiunit activity is absent in MT_1 receptor knockout mice (Liu et al., 1997). Expression of the MT_1 receptor has been reported in the human SCN (Weaver and Reppert, 1996).

It is interesting to note that while the acute inhibitory effects of melatonin on SCN multiunit activity are abolished in MT_1 melatonin receptor knockout mice, the phase shifting responses persist showing the existence of another distinct melatonin receptor (i.e., the MT_2 receptor, Liu et al., 1997). By contrast, in MT_2 receptor knockout mice the phase shifting response to melatonin was blunted while the inhibitory effect on firing of SCN neurons persisted (Jin et al., 2003).

An understanding of the mechanisms of melatonin receptor regulation in target tissues is an essential prerequisite for elucidating the role of endogenous and exogenously administered melatonin. A diurnal rhythm in the density of high-affinity receptors for melatonin, which is inversely related to its circulating levels, was first observed in hamster and rat brain membranes (Vacas and Cardinali, 1979) and in membranes prepared from rat hypothalamic sections containing the SCN (Laudon and Zisapel, 1986; Tenn and Niles, 1993). Thus, high-affinity binding was most pronounced in the latter part of the light phase, following prolonged depletion of the endogenous agonist, and least evident during darkness when exposure to elevated melatonin concentrations presumably down-regulated the high-affinity receptor (Vacas and Cardinali, 1979; Laudon and Zisapel, 1986; Tenn and Niles, 1993). A significant increase in high-affinity binding has been observed in the PT/median eminence of rats killed at the end of the light phase (when melatonin levels are low) as compared with animals killed in the morning (Vanecek et al., 1990).

Suppression or depletion of circulating melatonin levels by exposure to constant light or pinealectomy causes a significant increase in the density of high-affinity sites in rat brain membranes (Cardinali and Vacas, 1980) as well as in the pars tuberalis of the rat and hamster (Gauer et al., 1992). Conversely, a single injection of melatonin reverses the effect of constant light or pinealectomy on high-affinity binding in the rat pars tuberalis and SCN (Gauer et al., 1993). Moreover, preincubation of cultured ovine PT cells in the presence of melatonin (100 pM or 1 μM) has been found to significantly reduce melatonin binding in crude PT membranes (Hazlerigg et al., 1993). These early binding site studies did not identify the particular melatonin receptor subtype involved.

Pretreatment with physiological concentrations of melatonin (30–300 pM) decreased the number of MT_2 receptors in SCN (Gerdin et al., 2004b). The decrease in MT_2 melatonin receptor number induced by melatonin was reversible and regained full recovery after 8 h. The desensitization of MT_2 receptors in the SCN did not affect the desensitization of MT_1 receptors (Gerdin et al., 2004a).

Agonist-induced receptor desensitization is a normal process in the regulation of signal transduction in G protein-coupled receptors. Indeed, MT_1 receptors expressed in mouse hypothalamic GT1-7 neurons were desensitized following exposure to melatonin (10 nM) through a β-arrestin 1-dependent mechanism (Roy et al., 2001). In cells expressing the cloned MT_1 and MT_2 receptors, desensitization has been observed in both melatonin receptor subtypes and was associated with C-terminal phosphorylation and internalization suppression (MacKenzie et al., 2002; Witt-Enderby et al., 2004). A more recent study conducted in immortalized SCN cells indicated strong desensitization of the MT_2 receptor with physiological concentrations of melatonin (30–300 pM) (Gerdin et al., 2004a,b). This melatonin-induced down-regulation and desensitization in MT_2 receptors was reversible, with receptor density fully recovering after 8 h. Under similar conditions the authors reported desensitization of MT_1 receptors in the immortalized SCN cells, which was not accompanied by down-regulation of MT_1 receptors (Gerdin et al., 2004b). Prevention of melatonin-induced receptor desensitization has been demonstrated by experimentally depolymerizing microtubules in MT_1 receptor-expressing CHO cells and by preventing internalization and allowing reactivation at the membrane by protein phosphatases. The effectiveness of this procedure has been shown by its association with increases in the number of high affinity binding sites, their actions on G_i proteins and their impact on downstream PKC signaling cascades (Jarzynka et al., 2006). The desensitization of melatonin receptors in the SCN presumably plays a role in determining the temporal sensitivity profile of the mammalian biological clock to the melatonin signal. However, these studies were conducted in highly artificial systems, e.g., in CHO or GT1-7 cells transfected with the human melatonin receptor clones. A central issue therefore is whether the dynamics of desensitization which have been observed in vitro are the same as those occurring physiologically, and especially in the SCN.

These findings may be particularly relevant to the treatment of circadian rhythm sleep disorders (CRSD), which often depend on the administration of melatonin at pharmacological doses. Such dosage regimens can result in supraphysiological concentrations in the blood and thereby alter the functional sensitivity of melatonin MT_1/MT_2 receptors (Gerdin et al., 2004a). It should, however, be mentioned that the level of expression of melatonin receptors in the SCN is very low during the day, when melatonin levels are low, and high at night, when melatonin concentrations peak (Masana et al., 2000). Hence the increases in melatonin receptor concentration parallels the increases in the level of its ligand, suggesting that the regulation of the receptor level and sensitivity by melatonin is somewhat more complex than earlier studies had concluded. It must also be noted that there is no clinical evidence for desensitization of melatonin receptors upon use of supraphysiological doses of melatonin. Indeed, treatment with the melatonin receptor agonist S20098 for 14 days did not alter the sensitivity of photically responsive SCN cells to melatonin in the SCN, neither in vivo (intraperitoneal or iontophoretic application of melatonin) nor in SCN slices in vitro (Ying et al., 1998).

Paradoxically, a light pulse delivered during the subjective night (expected to halt melatonin secretion) decreased the specific binding of radiolabeled melatonin to MT_1 receptors in the SCN (Masson-Pevet et al., 1996, 2000). This observation is actually in line with findings of loss of MT_1 SCN receptors in conditions in which the endogenous melatonin output decreases, including cases of elderly patients and in those with AD (Wu and Swaab, 2007). Indeed, this decline in melatonin binding sites was noted in early studies on the aging rat hypothalamus (Laudon et al., 1988). The available evidence supports the conclusion that melatonin's regulation of melatonin receptors depends upon a number of

factors including the cellular milieu, the time of exposure, and the concentration of melatonin and the type of receptor considered (Gerdin et al., 2003).

Melatonin is the prototype of a "*chronobiotic*" (a substance that adjusts the timing or reinforces oscillators of the central biological clock). Lewy et al. (1992) were the first to report that administration of 2 mg of melatonin advances endogenous circadian rhythms in humans. Since then many investigations have confirmed that exogenous melatonin administration alters the timing of bodily rhythms, including sleep, core body temperature, or endogenous melatonin or cortisol rhythms (Arendt and Skene, 2005; Cardinali et al., 2006). Phase delays are observed with morning administration of melatonin, while phase advances are found after evening administration (Lewy et al., 1992). As part of its time signaling effect melatonin can induce opposing physiological changes in the body. Thus the organism's endogenous phase should be taken into consideration when analyzing melatonin's effects on target organs.

In recent years, clinical evidence has shown that exogenous melatonin administration is useful for treating a variety of circadian disruptions associated with, e.g., jet lag (Herxheimer, 2005), night shift work (Arendt and Skene, 2005), seasonal affective disorder (Lewy et al., 1998), delayed sleep phase syndrome (Dahlitz et al., 1991; Nagtegaal et al., 2000), and non-24-h circadian rhythm sleep disorder in the blind (Sack et al., 2000) and aging (Zisapel, 2007). The effects of melatonin on the SCN are pertinent to its clinical efficacy in CSRD [for reviews, see Zisapel, 2001a; Srinivasan et al., 2006b,c].

The development of non-selective (MT_1/MT_2) melatonin receptor agonists such as ramelteon (Rozerem™) (Roth et al., 2005; Pandi-Perumal et al., 2007) or agomelatine (Valdoxan™) (Dubocovich, 2006; Pandi-Perumal et al., 2006a) in addition to a prolonged release melatonin preparation (Circadin™) (Garfinkel et al., 1995; Leger et al., 2004; Lemoine et al., 2007; Wade et al., 2007) has further demonstrated that melatonin receptors play an important role in sleep regulation. Circadin improves quality of sleep, morning alertness, sleep onset latency and quality of life in middle aged and elderly insomnia patients (Garfinkel et al., 1995; Lemoine et al., 2007; Wade et al., 2007).

Ramelteon's affinity for MT_1 and MT_2 receptors is in fact greater than that of melatonin. In functional trials in patients with primary insomnia, ramelteon treatment significantly improved sleep onset latency (Roth et al., 2005, 2006). It is interesting, nevertheless, that the dose required for ramelteon to elicit its clinical effects in patients with insomnia (8–16 mg) is about 4–8 times higher than the 2 mg dose of melatonin used for the same purpose (Haimov et al., 1995; Garfinkel et al., 1995; Wade et al., 2007).

Agomelatine is a naphthalenic compound with strong melatoninergic properties. In accordance with its properties as a melatoninergic agonist, acting on both MT_1 and MT_2 receptors, it exhibits actions similar to those of melatonin, especially on the electrical activity of SCN neurons (Ying et al., 1996). This is in accordance with the properties of a melatoninergic agonist acting on both MT_1 and MT_2 receptors; additionally, agomelatine acts as a $5\text{-}HT_{2C}$ antagonist (Loo et al., 2002; Millan, 2005; Delagrange and Boutin, 2006). Agomelatine's effects on circadian phase (Leproult et al., 2005) and sleep have been described in man (Zupancic and Guilleminault, 2006). As will be further discussed below, the sleep regulating mechanisms of action of melatoninergic agents may involve action at the level of the SCN but also other brain areas such as the hippocampal and cortical areas.

5. Melatonin receptors in the eye

In the eye, melatonin is synthesized by a metabolic pathway which is similar to that which occurs in the pineal gland. The first description of melatonin biosynthetic capacity in the mammalian retina was made in 1971 by Cardinali and Rosner (1971a,b), who described the presence of HIOMT activity and the conversion of labeled serotonin into melatonin by the rat retina. Subsequent studies confirmed and extended those observations (Tosini and Menaker, 1998; Liu et al., 2004). The presence of HIOMT in the retina at both protein and mRNA levels has been documented in most vertebrates (Bernard et al., 1999). Melatonin exhibits a diurnal rhythm with maximal levels occurring during the dark phase. AANAT has been localized in rat retinal photoreceptors and shown to exhibit a daily rhythm with maximal expression at night (Niki et al., 1998; Iuvone et al., 2002). Although AANAT expression is a conserved feature of the vertebrate retina, the analyses of rhesus monkey and human retinas indicated that HIOMT protein and activity are very low and that HIOMT mRNA is detectable only by the most highly sensitive methodology (Rodriguez et al., 1994; Bernard et al., 1995; Coon et al., 2002). This has led to speculation that circulating melatonin or locally synthesized *N*-acetylserotonin may substitute for melatonin as a local signal in primate retina.

Immunocytochemical analysis of ocular tissues obtained from avian, rat and human sources has shown that MT_1 and MT_2 receptors are distributed in the cornea, choroid, sclera, photoreceptors, RGCs, retinal pigment epithelium (RPE) and retinal blood vessels (Fujieda et al., 1999; Scher et al., 2002; Wiechmann and Rada, 2003; Rada and Wiechmann, 2006). MT_1 receptors have also been identified in the corneal epithelium, stroma, sclera, and endothelium of *Xenopus* eyes (Wiechmann and Rada, 2003). The expression of MT_1 receptors in photoreceptor cells in the human eye provides evidence that melatonin acts directly on photoreceptors in mammals, particularly in the rod phototransduction pathway (Scher et al., 2002). The pervasive expression of melatonin receptors in ocular tissues generally suggests that melatonin plays a role in the differential regulation of the growth and remodeling of the fibrous and cartilaginous scleral layers which in turn affect eye size and refraction (Rada and Wiechmann, 2006). Melatonin receptors in the eye also play an important role in regulating aqueous humor secretion and the maintenance of the circadian variations of intraocular pressure (Osborne and Chidlow, 1994).

Melatonin MT_1 receptors are expressed in horizontal, amacrine and ganglion cells of the human retina suggesting that these cells are target sites for melatonin's actions (Scher et al., 2002). The expression of MT_1 receptors in most DA-containing amacrine cells in human retina implicates melatonin in the modulation of DA function by its direct action on neurons which participate in the rod phototransduction pathway (Scher et al., 2003). The expression of MT_1 receptors in the AII subtype of more than 50 subtypes of amacrine cells is evidence that melatonin has a specific physiological role in regulating retinal DA release (Fujieda et al., 2000). In the mammalian retina, the rod photoreceptors make synaptic connections with AII amacrine cells. These cells account for nearly 11% of total amacrine cells present in the retina (Strettoi and Masland, 1996). The modulation of AII gap junctions by ambient illumination forms the basis for visual acuity for the whole 24-h period, during which the retina is exposed to varying levels of illumination.

Retinal DA has been demonstrated to inhibit retinal melatonin synthesis (Nguyen-Legros et al., 1996; Tosini and Dirden, 2000; Jaliffa et al., 2000). Its effect is mediated through $D_{2/4}$ receptors that cause inhibition of AANAT activity. Melatonin, in turn, inhibits retinal DA synthesis (Dubocovich, 1984; Jaliffa et al., 2000). Indeed, DA and melatonin may act as mutually inhibitory retinal signals for day and night.

DA in the retina is essential for modulating AII receptive fields in light adapted retina (Hampson et al., 1992). DA modulates AII

receptive fields by uncoupling homologous AII junctions acting through D_1 receptors. Melatonin blocks this D_1 induced accumulation of cAMP via MT_1 receptors present in AII cells (Iuvone and Gan, 1995). MT_1 receptors are also expressed in some horizontal cells of the human retina (Scher et al., 2002) as well as in ocular vessels and in the adventia of retinal vessels. It has been suggested that this wide-ranging distribution of MT_1 receptors is indicative of melatonin's indirect action on vascular smooth muscle (Savaskan et al., 2002b).

Melatonin MT_2 receptors are also highly expressed in the human retina (Reppert et al., 1995). In addition, they are present in the sclera, lens and RPE (Wiechmann et al., 2004). The presence of MT_2 receptors in the apical microvilli of cell membrane, but not in the basal membrane of the RPE, suggests that melatonin is involved in photoreceptor outer segment disk shedding and phagocytosis (Wiechmann and Rada, 2003).

"MT_3 receptor" sites related to the quinone reductase 2 enzyme family have been identified in the eye and may play a role in the regulation of intraocular pressure (Pintor et al., 2001, 2003). For reviews on the possible involvement of melatonin in eye diseases, particularly in glaucoma, see Lundmark et al. (2006, 2007).

Recent data indicate a decrease in retinal MT_2 melatonin receptors expression in patients with AD (Savaskan et al., 2007). Whether this decrease is linked to the decrease in circulating melatonin production in these patients or functional disconnection from the SCN remains to be determined (Wu et al., 2007).

6. Melatonin receptors in the hippocampus

Melatonin binding sites exist in the hippocampus of several mammals. MT_1 and MT_2 receptors were localized in the dentate gyrus, CA3 and CA1 regions and subiculum of the hippocampus (Musshoff et al., 2002). Melatonin (1 μM) has been found to enhance the firing rate of neurons in the CA1 regions, an effect suppressed by the simultaneous administration of the MT_2 receptor antagonist luzindole (Musshoff et al., 2002). In murine hippocampal slices, melatonin induced a concentration-dependent inhibition of long-term potentiation, an effect prevented by the melatonin MT_2 receptor antagonists luzindole and 4-phenyl-2-propionamidotetraline (Wang et al., 2005). The inhibitory actions of melatonin were lost in mice deficient in MT_2 receptors but not those deficient in MT_1 receptors. Inasmuch as the inhibitory effect of melatonin was overcome by the application of forskolin, an activator of adenylyl cyclase, it was suggested that MT_2 receptors in the hippocampus were involved in the melatonin's action and that this action was mediated through the regulation of protein kinase A pathway (Wang et al., 2005). Recently, the functional consequences of MT_2 receptor deficiency was evaluated in MT_2 receptor knockout mice through the use of the elevated plus-maze paradigm (Larson et al., 2006). The learning/memory processes were impaired in MT_2 knockout mice suggesting that in these animals long-term synaptic plasticity in the hippocampus is altered (Larson et al., 2006).

Interestingly, in a randomized, double-blind, placebo controlled functional magnetic resonance imaging study of normal human subjects, melatonin enhanced the activation of the left parahippocampus in an autobiographic memory task (Gorfine et al., 2006). In a subsequent study, it was demonstrated that activation in the left hippocampus at 22:00 h was significantly reduced compared to afternoon hours compatible with diurnal variation in hippocampal activity (Gorfine and Zisapel, 2007). Exogenous melatonin further reduced activation in this region, but only in subjects who had already crossed the melatonin onset phase at this hour and in correlation with endogenous melatonin levels. Inasmuch as such an effect was not demonstrated with melatonin administration in the afternoon, a time dependent effect was suggested. Conversely, activation in the left parahippocampus at 22:00 h was greater in subjects who had passed the melatonin onset phase. Parahippocampal activation correlated with individual endogenous melatonin levels and was not further affected by exogenous melatonin. These results demonstrate that memory related activation in the hippocampus and parahippocampus are affected by time of day and melatonin in a differential manner and may implicate the circadian clock and melatonin in human memory processing during the night (Gorfine and Zisapel, 2007). Furthermore, melatonin appears to be involved in certain aspects of memory processing. In related brain imaging studies, Gorfine et al. (2007) found that the exogenous administration of melatonin was as effective as a 2 h mid-day nap for enhancing performance in a verbal association task, thus demonstrating that melatonin has an important role in memory consolidation.

In an immunohistochemical study of the brains of elderly human subjects and AD patients, Savaskan et al. (2002a, 2005) found a distinct increase in MT_1 melatonin receptor density in the hippocampus of AD patients that was localized in CA-1-4 subfields. The authors suggested that the increase was due to receptor up-regulation as a compensatory response to the impaired melatonin secretion seen in AD patients. By contrast, hippocampal MT_2 melatonin receptors, that in normal subjects are localized in pyramidal neurons of the human hippocampal subfields CA1-4 and in some granular neurons of the stratum granulosum, were decreased in AD patients, a finding that was interpreted as indicating that MT_2 receptors are involved in the mediation of melatonin action in the human hippocampus (Savaskan et al., 2005). The alterations of melatonin MT_2 receptor density in AD patients suggests that these receptors can be involved in the pathology of this disease whose prominent feature is cognitive impairment. The significance of melatonin in AD and its possible therapeutic applications are discussed elsewhere (Srinivasan et al., 2006a).

It is interesting to note that a moderate but significant improvement in cognitive function was documented in AD patients in placebo-controlled (Asayama et al., 2003) and open studies (Brusco et al., 1998a,b). In a retrospective study of the initial and final neuropsychological assessment of 50 outpatients diagnosed as having mild cognitive impairment, 25 of whom had received daily 3–9 mg of a fast-release melatonin preparation p.o. at bedtime for 9–18 months, patients treated with melatonin showed significantly better cognitive performance and a decrease in abnormally high depression scores. Concomitantly they showed improvements in wakefulness and sleep quality (Furio et al., 2007). These results suggest that melatonin can be a useful add-on drug for treating AD and mild cognitive impairment in a clinic environment.

It has been suggested that the loss of hippocampal MT_2 receptors could disturb the activity of γ-aminobutyric acid (GABA)-ergic transmission which may underlie the pathogenesis of AD. Administration of melatonin disrupted the inhibitory neuronal functions by reducing the $GABA_A$ receptor mediated currents in CA1 pyramidal cells through activation of MT_2 receptors (Musshoff et al., 2002). While melatonin does not interact with $GABA_A$ receptors, it is interesting to note that it potentiates the hypnotic effects of benzodiazepine and non-benzodiazepine drugs that enhance $GABA_A$ receptors (Ferini-Strambi et al., 1995; Garfinkel et al., 1997; Wesensten et al., 2005). Co-administration of melatonin during the withdrawal period has been shown to facilitate discontinuation of hypnotic drugs (Garfinkel et al., 1999; Siegrist et al., 2001).

7. Melatonin receptors in other brain areas

MT_1 and MT_2 receptors are expressed in the cell bodies of granule cells and basket-stellate cells of the human cerebellar

cortex and have been found to be co-localized with glutamatergic synapses (Mazzucchelli et al., 1996; Al Ghoul et al., 1998). The possible significance of melatonin for posture control and balance has been discussed (Fraschini et al., 1999).

MT_1 receptors have been localized in other areas of the brain such as the prefrontal cortex, caudate-putamen, substantia nigra, ventral tegmental area, nucleus accumbens and amygdala (Uz et al., 2005). MT_1 receptors exhibit diurnal rhythm with high protein levels occurring at night and low levels during the day. It is interesting that in the study by Uz et al. (2005) most areas described in rodents and humans that expressed MT_1 receptors were linked to the central dopaminergic pathways. These pathways, which are important for movement and reward systems, propagate through regions located in the midbrain (i.e., ventral tegmental area and substantia nigra) and forebrain (i.e., caudate nucleus, putamen, nucleus accumbens and prefrontal cortex) and constitute the nigro-striatal and mesolimbic pathways. These findings are in line with effects of melatonin on dopaminergic transmission in rodents [for review, see Zisapel, 2001b].

The existence of MT_1 receptors in nigro-striatal and mesolimbic pathways explains melatonin's efficacy in regulating dopaminergic behavior, such as the hypokinesia induced in rats by the D_2 antagonist fluphenazine (Sumaya et al., 2004). A single injection of melatonin markedly augmented cocaine-induced locomotor activity in rats while daily injections of melatonin blocked cocaine-induced behavioral sensitization (Sircar, 2000).

In a study of young rats which had been treated with amphetamines, melatonin was found to produce an inhibition of DA release and a significant increase in glutamate and aspartate release in the anterior hypothalamus. In middle-aged and aged rats, the inhibitory effects of melatonin on amphetamine-evoked DA release were maintained, but no effects on glutamate or aspartate release were found (Exposito et al., 1995). These results suggest that, during aging, the modulatory effect of melatonin on DA release in rat anterior hypothalamus is preserved whereas the DA-glutamate interaction is disrupted (Exposito et al., 1995).

Zhdanova and Giorgetti (2002) studied melatonin's effects on cocaine-induced anxiety-like behavior and cAMP levels in the nucleus accumbens of rats using a defensive withdrawal paradigm. Forty-eight hour after the last injection of cocaine, the rats that drank a solution of melatonin (200 ng/ml) at night, either during repeated cocaine administration or during its withdrawal, showed less anxiety-like behavior than controls. Further, the augmentation cAMP levels in the nucleus accumbens of melatonin treated rats was significantly attenuated (Zhdanova and Giorgetti, 2002). It has been suggested that melatonin and its analogues may regulate cocaine-induced behaviors through melatonin receptors located in areas responsible for the rewarding effects of drugs of abuse, such as the nucleus accumbens and the ventral tegmental area (Uz et al., 2005).

As mentioned above, quinone reductase 2 binds melatonin with high affinity (Nosjean et al., 2000) and has a specific pharmacological profile with affinity for prazosin and *N*-acetylserotonin in the brains of Syrian hamsters (the "MT_3 receptor") (Dubocovich et al., 2000). It has been suggested that elevated quinone reductase 2 activity may predispose individuals to neurodegenerative disorders such as Parkinson's disease (Delagrange and Boutin, 2006). Melatonin is useful in ameliorating certain symptoms of Parkinson's disease (Dowling et al., 2005) and since it inhibits quinone reductase 2 activity at low micromolar concentrations, its effectiveness could be linked to interaction with this enzyme, otherwise involved in free radical metabolism.

Numerous studies have suggested that a common comorbidity of depression is the abnormality of the circadian timing system. This seems to occur in various types of depression, but is particularly evident in seasonal affective disorder (Wehr and Goodwin, 1979). In nearly 80% of depressed patients, profound disturbances in sleep architecture have been documented (Wehr and Goodwin, 1979; Reynolds and Kupler, 1988; Armitage and Hoffmann, 2001). Melatonin has been implicated in mood disorders, particularly in seasonal affective disorder (Srinivasan et al., 2006c). In an investigation of the neurobehavioral consequences of missing the MT_1 receptor, MT_1 knockout ($MT_1-/-$) and wild-type mice were tested in the acoustic startle/prepulse inhibition, open field and Porsolt forced swim tests (Weil et al., 2006). The results indicated that lack of MT_1 signaling contributes to behavioral abnormalities including impairments in sensorimotor gating (a finding common in schizophrenia) and increases in depression-like behavior. This suggests that activity mediated by the MT_1 receptor may be at least partially responsible for normal brain and behavioral function. Consistent with the findings of Weil et al. (2006) on MT_1 knockout mice, Overstreet et al. (1998) found that melatonin produces behavioral enhancements in the forced swim test, suggesting that it may have antidepressant action.

Further supporting the inference that melatonin can have a role in alleviating depression, Imbesi et al. (2006) found that the CNS content of melatonin receptor mRNA was significantly modified by prolonged treatment with antidepressants such as fluoxetine, desipramine and clomipramine. The melatonin receptor agonist agomelatine functions additionally as a $5\text{-}HT_{2C}$ antagonist (Loo et al., 2002; Millan, 2005; Delagrange and Boutin, 2006) and the findings are consistent with clinical evidence showing that agomelatine has anxiolytic (Millan, 2005) and antidepressant (Dubocovich, 2006; Pandi-Perumal et al., 2006a) properties. It is interesting to note that while a link between melatonin and depression has not been consistently demonstrated, melatonin administration significantly improved the quality of sleep and vitality in adults exhibiting subsyndromal seasonal affective disorder (Leppamaki et al., 2003). Melatonin may be used to alleviate depressed mood during winter depression (Lewy et al., 1998). Co-administration of melatonin improves sleep in depressed patients undergoing therapy with serotonin reuptake inhibitors (Fainstein et al., 1997; Dolberg et al., 1998).

Several members of the G protein-coupled receptors family modulate the function of other receptors through heterodimerization. G protein receptor (GPR)50, an orphan G protein-coupled receptor, is about 45% identical to human MT_1 and MT_2 and heterodimerizes constitutively and specifically with MT_1 and MT_2 melatonin receptors in intact cells (Levoye et al., 2006). While the association between GPR50 and MT_2 did not modify MT_2 function, GPR50 abolished high-affinity agonist binding and G protein coupling to the MT_1 receptor. Interestingly, a genetic study has shown that a deletion mutant of GPR50 is associated with bipolar depression and major depressive disorder (Thomson et al., 2005). The relationship between melatonin receptors and affective disorders warrants further research.

8. Melatonin receptors in the cardiovascular system

Patients with coronary heart disease and non-dipper hypertensive patients (patients who do not exhibit a normal decline in blood pressure at night) have significantly lower nocturnal melatonin secretion than healthy controls (Brugger et al., 1995; Girotti et al., 2000; Yaprak et al., 2003; Jonas et al., 2003). Melatonin receptors (both MT_1 and MT_2) have been identified in the human coronary arteries of healthy human subjects and patients suffering from coronary heart disease (Ekmekcioglu et al., 2001a, 2003). Expression of MT_1 receptors in the coronary arteries exhibits 24 h variation with lowest values detected after 02:00 h

up to the late morning hours and a progressive increase seen after 13:00 h until midnight (Ekmekcioglu et al., 2001b). The expression of melatonin MT_1 receptor in the coronary arteries followed a similar rhythm and amplitude in coronary patients and in controls (Ekmekcioglu et al., 2001b). In contrast, melatonin MT_2 receptor expression in coronary arteries, aorta and left ventricles of patients with coronary heart disease differed from that of controls (Ekmekcioglu et al., 2003).

The role of melatonin in human coronary arteries is under intense investigation. Studies undertaken in animals have indicated that melatonin has dual effects on the vasculature with vasoconstriction being observed through MT_1 receptor activation, and vasodilatation through MT_2 receptor activation (Masana et al., 2002; Ersahin et al., 2002). The presence of MT_1 and MT_2 receptors has also been demonstrated in human left ventricular tissue (Ekmekcioglu et al., 2001a, 2003) but melatonin's role in left ventricular function has not been fully clarified. Melatonin stimulates high voltage activated calcium currents in embryonic heart tissues (Mei et al., 2001).

The finding that melatonin receptors in arteries are involved in thermoregulation was first demonstrated by Viswanathan et al. (1990) in rodents. The effect of melatonin on systemic vessels linked to thermoregulation was also demonstrated in humans inasmuch as the administration of melatonin during the day decreased core body temperature via vasodilatation in distal body regions (Krauchi et al., 1997; van den Heuvel et al., 1999). Exactly which melatonin receptors are involved in these effects remains to be investigated.

Melatonin is effective for lowering blood pressure in humans. In normal men (Arangino et al., 1999) and postmenopausal women undergoing hormone replacement therapy (Cagnacci et al., 2001), 1 mg melatonin p.o. reduced mean arterial blood pressure by about 6 mmHg. A decline in blood pressure after melatonin was also found in type 1 diabetic adolescents (Cavallo et al., 2004), essential hypertensive patients (Scheer et al., 2004) and in patients with nocturnal hypertension (Grossman et al., 2006). The melatonin receptor involved in this effect remains to be identified.

9. Melatonin receptors in the immune system

Melatonin is immunomodulatory in both animals and in humans (Maestroni, 2001; Srinivasan et al., 2005). Inhibition of melatonin synthesis for instance suppresses both cellular and humoral responses in mice (Maestroni et al., 1986).

The immunomodulatory role of melatonin is related in part to its action on specific melatonin receptors located in immunocompetent cells (Maestroni et al., 2002). Melatonin also regulates hematopoiesis through its action on specific receptors on bone marrow cells (Maestroni, 2001). In a study of human lymphocytic cells (Jurkat) and monocytic cells (U937), melatonin was found to enhance IL-2 and IL-6 production by acting through nuclear RZR/ROR α receptors and membrane MT_1 receptors (Garcia-Maurino et al., 2000). Inasmuch as melatonin stimulated IL-6 production from U937 monocyte cells when treated with IFN-γ (a procedure that induces the expression of the ROR γ1 and ROR α2 nuclear receptors and represses the expression of MT_1 receptor) Garcia-Maurino et al. (2000) concluded that the expression of nuclear melatonin receptor is essential for melatonin's effect on IL-6 production.

The contribution of MT_1 and MT_2 receptors in mediating the melatonin-induced enhancement of cellular and humoral immune function was explored in mice (Drazen and Nelson, 2001; Drazen et al., 2001). Melatonin enhanced splenocyte proliferation in both wild type and $MT_1-/-$ mice, an effect that was blocked by the MT_2 antagonist luzindole. This indicates that the melatonin-induced enhancement of immune function is mediated via MT_2 receptors (Drazen and Nelson, 2001; Drazen et al., 2001).

In the thymus and spleen of mice, a significant expression of membrane MT_1 and MT_2 receptors, and of nuclear RZR/ROR α, occurs (Carrillo-Vico et al., 2003). The use of the MT_2 receptor blocker luzindole or of the nuclear RZR/ROR blocker CGP 55644 has been found to reduce human lymphocyte IL-2 and IL-2 receptor production (Carrillo-Vico et al., 2004). Indeed, human lymphocyte-synthesized melatonin may play a crucial role in modulating the IL-2/IL-2 receptor system. This conclusion is based on a study showing that when melatonin biosynthesis is blocked by the tryptophan hydroxylase inhibitor parachlorophenylalanine, both IL-2 and IL-2 receptor levels fall, an effect counteracted by the addition of exogenous melatonin (Carrillo-Vico et al., 2005). Similarly, prostaglandin E_2-induced inhibition on IL-2 production increased when MT_2 membrane receptors were blocked by luzindole (Carrillo-Vico et al., 2005). Taken together, these data indicate that melatonin synthesized in human lymphocytes is involved in the physiological regulation of the IL-2/IL-2R expression through a mechanism comprising both membrane and nuclear melatonin receptors (Carrillo-Vico et al., 2006).

Immunosurveillance is one of the major phenomena through which cancer cells are detected and eliminated. The activation of lymphocytes and monocytes/macrophages by melatonin is one of the principal mechanisms by which melatonin prevents tumor development (Martins E Jr et al., 1998; Miller et al., 2006). It has been found that neoplasms resistant to IL-2 respond well to IL-2 therapy after the concomitant administration of melatonin. In one study, patients who received both IL-2 and melatonin exhibited a significantly higher number of lymphocytes, T lymphocytes, NK cells, and $CD4^+$ cells than those receiving IL-2 alone (Lissoni et al., 1993a). Immunotherapy with a low dose of IL-2 and melatonin was beneficial in treating advanced tumors of the digestive tract (Lissoni et al., 1993b). Melatonin's influence in enhancing IL-2 production by acting on human lymphocytes and in augmenting IL-6 production by acting through monocytic cells underscores the importance of melatonin receptors in the oncostasis exerted by melatonin (Lissoni, 1999). In addition to immunostimulation, the melatonin/IL-2 relationship may be particularly relevant for immune tolerance. $CD4^+$-$CD25^+$ T regulatory cells represent a critical T cell subset that suppresses peripheral autoreactive T cells that escape thymic negative selection (Malek and Bayer, 2004). IL 2-deficient mice exhibit a lethal lymphoproliferative disorder accompanied by severe autoimmunity (Suzuki et al., 1995). IL-2 and IL-2R knockout mice contain severely reduced numbers of $CD4^+$-$CD25^+$ cells (Malek and Bayer, 2004). The finding that normalizing the production of $CD4^+$-$CD25^+$ T regulatory cells in such mice can inhibit uncontrolled T cell proliferation and autoimmunity (Wolf et al., 2001) suggests that melatonin may affect T-cell tolerance via IL2. It is possible that the ability of melatonin to enhance inflammatory cytokine production from human monocytes/macrophages, including IL-6 and IL-12, may turn the melatonin/IL-2 connection toward the enhancement of T cell immunity rather than to the formation of T regulatory cells. It has in fact been reported that IL-6 plays a crucial role in driving the differentiation of T regulatory cells into IL-17 secreting T-helper cells which contribute in Th-1 and autoimmune responses (Xu et al., 2007). Thus, in the absence of inflammatory stimuli the immunological function of melatonin might be opposite to that exerted during inflammation and/or infection. Further studies are necessary to confirm these hypotheses.

10. Melatonin receptors in cancer cells: clinical implications

10.1. Breast cancer

Ever since Bartsch et al. (1981) showed that compared to healthy controls, Indian women with advanced breast cancer had

diminished urinary levels of melatonin, a number of functional studies have shown a relationship between melatonin and human breast cancer. Tamarkin et al. (1982) found that compared to age matched controls, the normal nocturnal increase in the plasma concentration of melatonin in women with estrogen-receptor (ER) positive breast tumors was significantly reduced and further that an inverse correlation existed between ER levels and peak melatonin values. It has been hypothesized that the circadian rhythm of nocturnal melatonin production may represent a "regulatory signal" for the carcinogenic process; it may exert a "natural restraint" on tumor initiation, promotion, and/or progression (Blask et al., 2005a,b).

In an in vitro study, Hill and Blask (1988) demonstrated that physiological concentrations of melatonin cannot only alter the morphology of estrogen-responsive MCF-7 human breast cancer but also inhibit the growth of cancer cells. Nanomolar concentration of melatonin in the presence of serum or estradiol: (i) inhibits, in a reversible way, cell proliferation (Cos et al., 1991), (ii) increases the expression of p53 and p21WAF1 proteins and modulates the length of the cell cycle (Mediavilla et al., 1999), and (iii) reduces the metastatic capacity of these cells and counteracts the stimulatory effect of estradiol on cell invasiveness (Cos et al., 1998). The latter effect is mediated, at least in part, by a melatonin-induced increase in the expression of the cell surface adhesion proteins E-cadherin and β_1-integrin.

The direct oncostatic effects of melatonin depend on its interaction with the tumor cell estrogen-responsive pathway since melatonin down-regulates the expression of ER α and inhibits the binding of the estradiol-ER complex to the estrogen response element in DNA (Lawson et al., 1992; Molis et al., 1994; Rato et al., 1999). Interestingly, estradiol inactivates melatonin receptors and consequently responses in human epithelial benign prostatic hyperplasia cells, probably through PKC activation (Gilad et al., 1997b). In the rat hypothalamus, estradiol was associated with higher affinity binding of ^{125}I-melatonin and higher inhibition by melatonin of stimulated DA release (Zisapel et al., 1987; Laudon and Zisapel, 1987).

The molecular mechanisms for the oncostatic actions of melatonin have been inferred from its actions on membrane MT_1 and MT_2 receptors and on nuclear RZR/ROR α receptors. A putative melatonin agonist CGP-52608 with specific binding to nuclear RZR/ROR α orphan receptors and no affinity for MT_1 and MT_2 receptors was used to study melatonin's action on MCF-7 human breast cancer cells. Similar to melatonin, CGP-52608 inhibited the MCF-7 cell proliferation in a time dependent manner (Ram et al., 2002). However, the dose of CGP-52608 used was found to be lethal for MCF-7 cells, suggesting that nuclear RZR/ROR α orphan receptors are not the primary receptors for mediating melatonin's oncostatic effects.

Since MCF-7 cells contain MT_1 receptors, the efficacy of the MT_1 agonist *N*-acetyl-4-aminomethyl-6-methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole (AMMTC) was tested at a concentration of 10^{-9} M. AMMTC significantly inhibited MCF-7 cell proliferation by 40% (Ram et al., 2002). Melatonin at a concentration of 1 nM caused a significant 33% and 21% inhibition of cell growth in MCF-7 and vt-MCF 7 cells (Yuan et al., 2002). Inasmuch as the melatonin and AMMTC growth inhibitory effect on MCF-7 was abrogated by specific MT_1 receptor antagonism, Ram et al. (2002) concluded that the MT_1 melatonin receptor is the primary mediator of melatonin's growth inhibitory effect on MCF-7 human breast cancer cells. Additional evidence for this conclusion was provided by the use of S20928, a MT_1/MT_2 melatonin G protein coupled receptor antagonist, which abolished melatonin's growth-suppressive effects (Yuan et al., 2002).

Overexpression of the MT_1 G protein coupled melatonin receptor also significantly inhibited the basal proliferative rate of ERα positive MCF-7 cells. This elevated expression of MT_1 receptors was associated with the increase in the radiolabeled melatonin binding and enhancement of the growth-inhibitory and gene modulatory effects of melatonin in ER α positive human breast cancer cells (Yuan et al., 2002).

The decrease in cAMP production caused by melatonin via MT_1 and MT_2 receptor interaction decreases the uptake of linoelic acid, an essential fatty acid, by specific fatty acid transporters (Blask et al., 2002). Linoleic acid can be oxidized to 13-hydroxyoctadecadienoic acid by 15-lipoxygenase, serving as an energy source for tumor growth and tumor growth signaling molecules. Inhibition of linoleic acid uptake by melatonin is regarded as a mechanism of its antiproliferative effects (Blask et al., 2002).

The inhibitory action of melatonin on mammary carcinogenesis has also been attributed to melatonin's immunomodulatory effects (Maestroni, 1993; Vijayalaxmi et al., 2002; Anisimov et al., 2006; Miller et al., 2006). Indeed, disturbances of immune mechanisms have been documented in experimental models of mammary cancer. For example, the absence of the cytosolic protein Nod1 in MCF-7 cells correlated with tumor growth, an increased sensitivity to estrogen induced cell proliferation, and a failure to undergo Nod1-dependent apoptosis (da Silva et al., 2006). IL (interleukin)-2 and chemotherapy are employed in the treatment of metastatic breast cancer (Miller et al., 1997; Gravis et al., 2000; Burns et al., 2003). IL-2 is used to increase the efficacy and cytolytic function of natural killer (NK) cells and, in combination with interferon and chemotherapy, has been used as an adjuvant treatment in high-risk breast cancer (Tonini et al., 1998). The link between melatonin and the immune system in cancer has been explored in a recent Phase II study with melatonin causing increases in some cytokines and amplifying the objective responses to cytokine in patients (Abrial et al., 2005).

Angiogenesis is an essential step in the development of primary tumors. Endothelin-1 synthesis in blood vessels is considered as one of the main stimulants of angiogenesis in primary tumors via the release of vascular endothelial growth factor, an effect that is arrested by melatonin which suppresses the formation of endothelin-1 by inhibiting endothelin-converting enzyme-1 (Kilic et al., 2004). Further characterization of this effect will be revealing about the molecular mechanisms by which melatonin causes vacuolization of tumors and will contribute significantly to the understanding of the anti-tumor effect of melatonin.

Collectively, these studies demonstrate the critical role of MT_1 receptors in mediating melatonin's antiproliferative effect on human breast cancer cells and point to the possibility that melatonin or its MT_1 analogs may have potential as agents for oncostatic therapy. Further studies are required, however, to determine if the effects observed in cultured cells can be extrapolated to whole living systems.

10.2. Ovarian and endometrial cancer

After the identification of melatonin in the ovary (Brzezinski et al., 1987; Ronnberg et al., 1990), several studies sought to examine the local role of melatonin. Melatonin modulates a number of physiological functions of the ovary by acting through MT_1 and MT_2 receptors (Masana and Dubocovich, 2001). Both receptors were detected in human (Niles et al., 1999) and murine granulose cells (Clemens et al., 2001).

Studies conducted on human ovarian cells (BG-1 cell line) in vitro have shown that melatonin (10^{-5} to 10^{-10} M) decreased proliferation of these cells by more than 20% (Petranka et al., 1999). A non-homogeneous response to melatonin was found in ovarian carcinoma cell cultures of different cell lines. Cells of one tumor line were inhibited by 90% at a melatonin concentration of 10^{-8} M,

while cells from a second tumor showed a growth inhibition by 30% (Bartsch et al., 2000b). In another study, 10^{-9} M melatonin exerted an anti-proliferative effect in OVCAR-3 cell lines (Futagami et al., 2001). Studies conducted in ovarian carcinoma patients did not show any significant difference in melatonin secretion as compared to controls (Bartsch et al., 2000a).

Endometrial carcinoma is the most common malignant neoplasm of the female genital tract. Although the pathogenesis of this malignancy is not well understood, melatonin deficiency was seen as one of the possible factors, due to its anti-estrogenic properties. A reduced melatonin secretion has been reported in patients with endometrial cancer (Karasek et al., 2000). It has been suggested that individual differences are involved in tumor growth modulation and sensitivity to melatonin (Bartsch et al., 2000a).

10.3. Colon cancer and hepatoma

Colorectal cancer is one of the leading types of cancers of the gastrointestinal tract. It is predicted that the prevalence of colorectal cancer will increase in the general population from 0.36% to 0.46% between year 2000 and 2020 (Mariotto et al., 2006). The presence of melatonin in the enterochromaffin cells of the gastrointestinal tract was first demonstrated by Raikhlin and Kvetnoy (1976). After systemic administration, melatonin was found to accumulate in the stomach and the colon (Bubenik, 2002). Maximum binding occurred in the mucosa and intestinal villi (Lee et al., 1995). Melatonin receptors are localized in the gastrointestinal system. The highest concentration of melatonin receptors is found in the mucosa of jejunum and colon (Pontoire et al., 1993; Lee and Pang, 1993; Poon et al., 1996). In the colon, melatonin is thought to play a role in regulating Cl^- secretion (Chan et al., 1998). A circadian variation in the expression of melatonin receptors has been described in the gastrointestinal tract, with maximum density of receptors occurring at 21:00 h coinciding with increased levels of circulating melatonin (Motilva et al., 2001).

Melatonin may modulate intestinal ion transport and motility (Bubenik, 2002). In the duodenal mucosa, melatonin influences HCO_3^- secretion from the duodenal mucosa through an effect mediated by MT_2 receptors (Sjoblom and Flemstrom, 2003; Sjoblom et al., 2003). In a recent study, the MT_1 receptor was localized in the intestinal segments of the rat with the highest transcript levels in the epithelial and subepithelial layers of the duodenum (Sotak et al., 2006). The expression of melatonin receptors was up-regulated by nutritional deprivation.

Interest in the possible role of melatonin in colorectal cancer increased following the identification of melatonin binding sites in human colon tissue from patients with carcinoma of the rectum or colon. ^{125}I-melatonin binding sites were identified in the mucosa/submucosa of the human colon and radioimmunoassay revealed melatonin concentration of 467 ± 99 pg/g human colon (Poon et al., 1996). Daytime melatonin concentration in the colon of patients with colorectal carcinoma was 3147 ± 87.8 pg/g tissue (Poon et al., 1996).

In line with the findings for breast cancer, i.e., that nocturnal lighting suppresses melatonin production, the fact of being employed on a rotating shift at least three nights per month for more than 15 years was found to increase significantly the risk of colorectal cancer in female nurses (Schernhammer et al., 2003). Further confirmation of a possible association between reduced melatonin output and cancer development was provided in a study of melatonin levels in patients with colorectal carcinoma of melatonin levels in 12 female and 21 male patients with colorectal carcinoma (Kos-Kudla et al., 2002). Compared to 28 healthy age-matched controls, the cancer patients showed significant decrements in the peak amplitude of melatonin secretion as well as a reduction in overall melatonin output (Kos-Kudla et al., 2002).

The effect of melatonin on inhibition of colon carcinoma cell growth was studied in CT-26 cells, a murine colon carcinoma-derived cell line (Farriol et al., 2000). Although melatonin had no effect on cell growth at low doses, a statistically significant and progressive suppression of DNA synthesis was found as melatonin doses increased (the percentage of inhibition being 1 mM, 22%; 2 mM 25%; 3 mM 47%) (Farriol et al., 2000). Inasmuch as no receptors were involved in the cell line selected, it was concluded that melatonin exerted its anti-proliferative action through a non-hormone dependent mechanism. In another study, the oncostatic effect of melatonin on colon cancer was demonstrated to be mediated through MT_2 receptors and through its binding to nuclear RZR/ROR α receptor (Winczyk et al., 2002). CGP 55644, an antagonist of the nuclear RZR/ROR α receptor, diminished the oncostatic effect of melatonin on murine colon.

The effect of the simultaneous administration of melatonin and the cytotoxic drug CPT-11 was evaluated in 30 patients with metastatic colorectal carcinoma (Cerea et al., 2003). It was found that co-administration of melatonin (20 mg/day at bedtime) with CPT-11 was more effective in controlling the disease than administration of CPT-11 alone. These findings support the inference that melatonin could enhance the effect of chemotherapy in colorectal carcinoma. By increasing the level of oxidizable substrates that can be incorporated into mitochondria in the presence of flavone, melatonin (1 mM concentration) has been shown to potentiate flavone-induced apoptosis in HT-29 human colon cancer cells (Wenzel et al., 2005).

Melatonin was detected in the human liver in a concentration that was 15 times higher than that in blood (Messner et al., 2001). In gallbladder bile, melatonin's concentration ranges from 2000 to 11,000 pg/ml, a concentration suitable for prevention of oxidative damage (Tan et al., 1999). Expression of MT_1 receptors also occurs in the epithelia of human gall bladder cells (Aust et al., 2004).

N-nitrosodiethylamine (NDEA) is a potent carcinogenic agent that induces liver cancer. In a recent study performed to evaluate the chemopreventive function of melatonin in this experimental model, Wistar male rats received a single i.p. injection of NDEA or vehicle followed by weekly s.c. injections of carbon tetrachloride or vehicle for 6 weeks. Melatonin (5 mg/kg body weight) or its vehicle (0.5 ml saline) was given i.p. on a daily basis 2 h before lights off for 20 weeks (Subramanian et al., 2007). At the end of this period the rats were sacrificed and liver and blood samples were taken for histological and biochemical studies. NDEA administration inhibited body weight, produced macro- and microscopically detectable liver tumors and increased levels of plasma aspartate transaminase, alanine transaminase and α-fetoprotein. NDEA treatment decreased the levels of liver thiobarbituric acid reactive substances and the activity of catalase and superoxide dismutase and increased liver reduced glutathione levels and glutathione peroxidase and glutathione *S*-transferase. Melatonin administration significantly curtailed tumor development and counteracted all the biochemical effects, presumably via its direct and indirect antioxidant effects (Subramanian et al., 2007).

Melatonin's oncostatic effect on hepatoma 7288CTC cell lines is due to its inhibition of fatty acid uptake, an action mediated via MT_1 receptors and a G_1 protein-coupled signal transduction pathway (Blask et al., 1999; Sauer et al., 2001). Melatonin's regulatory influence on lipid homeostasis in tumors via melatonin receptors is a key mechanism which underlies its oncostatic effects.

In a study conducted on 35 patients (colorectal cancer = 14; gastric cancer = 8; hepatic carcinoma = 6; pancreatic adenocarcinoma = 7), interleukin (IL)-2 and melatonin were administered

simultaneously (Lissoni et al., 1993a). Melatonin was given orally at a daily dose of 50 mg starting 7 days before IL-2, which was given simultaneously at a dose of 3 million IU/day at 20:00 h for 6 days a week for 4 weeks, which corresponds to one cycle of immunotherapy. Complete response was achieved in two patients (gastric cancer = 1, and hepatic carcinoma = 1). Partial response was shown in 6 other patients (gastric cancer = 2, hepatic carcinoma = 2, colon cancer = 1, pancreatic cancer = 1). The overall response rate was 8/35 (23%). The authors concluded from this study that a low dose of IL-2 plus melatonin can be well tolerated and holds a promise for an effective therapy of advanced tumors of the digestive tract (Lissoni et al., 1993a).

In another study on 100 patients with inoperable advanced primary hepatocellular carcinoma, trans-catheter arterial chemoembolization (TACE) was used along or associated with melatonin (Yan et al., 2002). The effectiveness rates of TACE or TACE + melatonin were 16% and 28% respectively. The survival rate of 0.5, 1 and 2 years in the TACE group was 82%, 54%, and 26% respectively, while in the TACE + melatonin group it was 100%, 68% and 40% respectively. An elevation of IL-2 level was noted in the patients treated in combination with melatonin, showing thereby that immunoenhancing function of melatonin contributed to a greater percentage of response in this group of patients (Yan et al., 2002).

Endogenous melatonin has protective effects in pancreatic tissue. This presumably occurs through its action on MT_2 receptors as suggested by the observation that the exogenous administration of luzindole aggravates caerulin-induced pancreatitis (Jaworek et al., 2002), often a precarcinoma lesion.

10.4. Melanoma

The MT_1 receptor is widely distributed in human skin; it is located in most cutaneous cells including the keratinocytes, melanocytes, fibroblasts squamous cell carcinoma and melanoma cells (Slominski et al., 2002). MT_1 immunoreactivity has also been detected in keratinocytes of the spinous and granular epidermal layers of the human scalp (Slominski et al., 2005). MT_2 receptors are found in normal and malignant melanocytes (Roberts et al., 2000) and in eccrine sweat glands (Slominski et al., 2005).

It has been suggested that the cutaneous melatoninergic system can protect the physical and functional integrity of the skin against environmental stress or internal dyshomeostatic stimuli. Melatonin protects human skin against UV radiation (Slominski et al., 2003) and X-rays (Kanikkannan et al., 2001). The radioprotective action of melatonin has been attributed mainly to its ability to scavenge free radicals and to stimulate antioxidative enzymes (Kim et al., 2001; Slominski et al., 2005). Specifically, melatonin has been implicated in hair growth cycling and cutaneous pigmentation (Slominski et al., 2004).

In human melanocytes and melanoma cells, melatonin and its membrane receptor agonists inhibit growth presumably by the activation of MT_2 receptors (Roberts et al., 2000). The anti-proliferative effect of melatonin in the human melanoma cell line SK-Mel 28 was also mediated via melatonin MT_2 receptors since it was reversed by the MT_2 receptor antagonist luzindole (Souza et al., 2003). Melatonin's anti-proliferative effect has also been demonstrated in B16 murine melanoma cells where it was found to be dose-dependent with higher concentrations being more inhibitory than the lower ones (Yerneni and Jayaraman, 2003). The effect of melatonin ranges from moderately inhibitory (oncostatic) to highly inhibitory to lethal (oncotoxic). It was reported that at low concentrations (2–77 pg/cell) melatonin did not significantly affect the cell number, but at intermediate concentrations (1936–2003 pg/cell) a moderate inhibitory effect $\geq$50% in comparison to control values was shown. At higher concentrations (7743–19,356 pg/cell) melatonin caused >50% drop in the cell number. The most striking finding was that at the highest concentration there was total elimination of cells by day 5 of the study. Such an effect of melatonin has been attributed to its direct effect of melatonin and not to a receptor-mediated cascade of reactions (Yerneni and Jayaraman, 2003).

In cultured human uveal melanoma cells, melatonin inhibited growth in a dose dependent manner in the range of concentrations 0.1–10 nM (Hu et al., 1998). In another study, the growth inhibitory effect of melatonin was demonstrated at a concentration of 2 nM, the physiological concentration found in aqueous humor; the growth inhibiting effect of melatonin on uveal melanoma cells was shown to be related to activation of MT_1 melatonin receptors (Roberts et al., 2000). In addition, the anti-proliferative effect of melatonin was attributed to the stimulatory action on antioxidative enzyme synthesis (Kadekaro et al., 2004).

High daytime serum melatonin levels (9.7–20.9 pg/ml) was reported in patients with melanoma as compared to normal individuals (1.4–2.1 pg/ml) (Kerenyi et al., 1990). High melatonin levels were reported in patients with choroidal melanoma where melatonin levels decreased following enucleation (Kiratli et al., 2003). The increase of melatonin levels seen in these patients was suggested as a compensatory mechanism for arresting the cancerous growth (Kiratli et al., 2003).

10.5. Prostate cancer

The rat prostate contains GTP-sensitive melatonin binding sites (Gilad et al., 1998b). Daily injections of testosterone to castrated rats induced regrowth of the prostate and increased the weight of the seminal vesicles. Administration of melatonin to the rats through drinking water prevented the testosterone-mediated regrowth of the prostate. Human prostate epithelial cells contain specific 2-^{125}I-melatonin binding sites that are involved in controlling cell growth and viability (Laudon et al., 1996; Gilad et al., 1996). These receptors enhance cAMP through pertussis toxin and inhibit cGMP through cholera toxin sensitive G proteins (Gilad et al., 1998b). MT_1 receptors have been localized in androgen sensitive LNCaP cells, a cell line whose growth is inhibited by melatonin, thus suggesting that the MT_1 expression is involved in the antitumor activity of melatonin (Lupowitz and Zisapel, 1999; Xi et al., 2001).

Melatonin receptors are also expressed in the androgen-independent PC3 human prostate carcinoma cells (Gilad et al., 1999). It has been suggested that the mechanism for melatonin's oncostatic action in inhibiting androgen induced prostate cell growth might be its ability to dislocate androgen receptors from the nucleus to the cytoplasm of prostate epithelial cells (Rimler et al., 2001, 2002a,b). This process is mediated via a Gi protein, an increase in cGMP, calcium entry and activation of PKC alpha subtype (Lupowitz et al., 2001; Sampson et al., 2006; Rimler et al., 2007). Activation of PKC alpha in the cells mediates intracellular trafficking of RGS10 and RGS4 proteins (Rimler et al., 2006, 2007) and inactivates melatonin receptors in human prostate epithelial cells (Gilad et al., 1997a). PKC activation and RGS proteins mobilization may underlie the unique ability of melatonin to affect multiple G proteins in cells. In this respect, it is interesting to note that knock-down of RGS4 in CHO cells expressing the human MT_1 melatonin receptor prevents melatonin-induced receptor desensitization (Witt-Enderby et al., 2004).

The MT_1 receptor was identified in prostate cancer patients and treatment with melatonin induced stabilization of this hormone-refractory disease (Shiu et al., 2003). The melatonin-mediated inhibition of androgen receptor mediated cancer cell growth may

explain the efficacy of gonadotropin-releasing hormone (GnRH) analogue plus melatonin in metastatic prostate cancer patients progressing on GnRH analogue alone (Lissoni et al., 1997).

11. Conclusions

Evidence obtained from a number of studies indicates that melatonin exerts its physiological action in many areas of the CNS, such as the SCN, hippocampus, dopaminergic pathways, prefrontal cortex and cerebellum, by acting through G protein coupled membrane MT_1 or MT_2 receptors. MT_1 and MT_2 receptors in the SCN and hippocampus and melatonin's physiological activities in these areas implicate these receptors in the regulation of sleep and circadian rhythms and perhaps memory consolidation. The alteration in expression of either MT_1 or MT_2 receptors in the brain of patients suffering from AD may be related to the pathogenesis of this neurodegenerative disease, but the hypothesis requires further confirmation by clinical and physiological studies. While melatonin receptor-mediated cAMP action in the mesolimbic dopaminergic system suggests that melatonin can be involved in the regulation of addictive behaviors, the physiopathological significance of melatonin receptors in parkinsonism and depressive disorders is still under investigation.

In the eye, MT_1 receptors are involved in phototransduction. The wide distribution of functional melatonin receptors in the eye points to a melatonin role in ocular growth and aqueous humor secretion, the latter action being of importance for glaucoma.

Melatonin receptors (both MT_1 and MT_2) have been demonstrated in the human coronary arteries of healthy human subjects and patients suffering from coronary heart disease. Additionally they have been found in peripheral blood vessels, presumably mediating the effectiveness of melatonin for lowering blood pressure in humans.

In MCF-7 breast cancer cells, MT_1 and MT_2 receptors and ROR α mediate melatonin's direct oncostatic effects, which are complemented by the immunomodulatory and antioxidant effects melatonin has in vivo. An oncostatic role of melatonin receptors in prostate cancer has also been proposed. Melatonin inhibits androgen receptor mediated effects through its exclusion from the cell nucleus.

In the immune system, membrane and nuclear melatonin receptors are all involved in the modulation of cell and humoral immunity. Melatonin's action in enhancing IL-2 and inflammatory cytokines production from lympho-monocytes through melatonin receptors is one of the mechanisms through which melatonin exerts its oncostatic action.

The distribution of melatoninergic receptors in the human skin has a protective role for the skin and there is some evidence to indicate that melatonin receptors are involved in controlling melanoma growth and the hair cycle.

The plethora of melatonin effects are mediated in part by G proteins coupled to melatonin receptors, but other G proteins and their downstream effectors may be linked to the effects of melatonin on the intracellular localization of RGS4 and RGS10 proteins, through the melatonin-mediated activation of PKC-α. These findings, which have been derived from both in vitro and physiological studies, provide preliminary support for the hypothesis that melatonin receptors may play a role in the regulation of many physiological processes in the organism. Other evidence has suggested that dysfunction of the melatoninergic system can facilitate the development of a number of adult onset diseases including CRSD, AD and Parkinson disease, glaucoma, depressive disorder, breast and prostate cancer, hepatoma, melanoma and hypertension. While the available evidence is suggestive that melatonin can prevent or ameliorate some symptoms of these diseases, this inference remains to be confirmed in further animal and clinical studies.

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