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Melatonin Madness

Minireview

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Melatonin madness is everywhere! The media attention that melatonin has received recently is truly remarkable and has elevated the hormone to cult status. Is all this excitement really justified, or is it just hype? What does melatonin actually do? Does it signal through specific receptors like other hormones? What is its true therapeutic potential? This minireview addresses these questions and highlights recent discoveries that increase our understanding of the cellular and molecular actions of this hormone.

Melatonin is the principal hormone of the vertebrate pineal gland. Pineal melatonin production exhibits a striking circadian rhythm that is reflected in circulating melatonin levels. In mammals, the melatonin rhythm is generated by a master circadian clock in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN is synchronized (entrained) to the 24 hr day by the daily light–dark cycle, with light signaling through a direct retinal pathway to SCN. The SCN clock sends circadian signals over a multisynaptic neural pathway to the pineal gland, driving rhythmic melatonin production. Within the pineal gland, melatonin is synthesized from serotonin (5-hydroxytryptamine [5-HT]). The rate-limiting step in melatonin production is the N-acetylation of 5-HT by arylalkylamine N-acetyltransferase (AA-NAT), an enzyme whose cDNA has been recently cloned (Coon et al., 1995). The AA-NAT rhythm is under SCN control, with the resulting melatonin rhythm distinguished by high levels at night in all vertebrates.

The "Melatonin Miracle" Is a Mirage

A centerpiece of the so-called melatonin miracle sensationalized in a recent pop science book is the claim that the hormone can reverse aging (Pierpaoli and Regelson, 1995). This assertion is scientifically unfounded and is based on the results of a seriously flawed study performed in mice (Pierpaoli and Regelson, 1994). All the murine strains used in that study (BALB/c, C57BL/6, and NZB) have a well-described genetic defect in pineal melatonin biosynthesis and cannot make melatonin (Goto et al., 1994, and references therein). Thus, any assertion that youthful pineal glands transplanted into old mice keep old mice from aging owing to restoration of "youthful" melatonin levels is absurd. When these melatonin-deficient strains of mice were treated with pharmacological doses of melatonin, there was a 20% increase in life span in female NZB and BALB/c mice, but not in female C57BL/6 mice. However, these authors showed in another study (that is generally overlooked) that when comparable amounts of melatonin are given to a mouse strain (C3H/He) that makes melatonin, the treatment actually shortened survival by inducing reproductive tract tumors (referenced by Pierpaoli and Regelson, 1994). Thus, there is no evidence that melatonin administered to melatonin-producing mice can increase longevity. The evidence that melatonin can increase longevity in mice in general is inconsistent. The suggestion that melatonin may increase longevity is humans is based purely on speculation.

The antioxidant effect of melatonin has also been embellished, leading to claims that melatonin is a wonder drug useful for treating everything from AIDS to Alzheimer's disease (Reiter and Robinson, 1995). Melatonin does act as an intracellular scavenger of hydroxyl and peroxyl free radicals when administered at pharmacological doses both in vivo and in vitro (reviewed by Reiter, 1995). Although the antioxidant potential of melatonin may have some therapeutic applications (although clearly not as many as claimed!), these antioxidant effects require melatonin concentrations about 10^6-fold greater than the physiological melatonin concentration (which is <1 nM). Thus, the antioxidant effects of melatonin, being pharmacological, would not be expected to be mediated through physiologically relevant high affinity receptors. To no great surprise, advocates of melatonin's antioxidant abilities have embraced with open arms the unsubstantiated claims that melatonin may signal through low affinity nuclear receptors of the retinoid Z receptor family (Becker-Andre et al., 1994; Wiesenberg et al., 1995).

Melatonin Has Specific Neurobiological Effects

Melatonin does have several bona fide biological effects that are mediated through high affinity receptors (Figure 1). The most well-documented physiological role of melatonin is its regulation of seasonal responses to changes

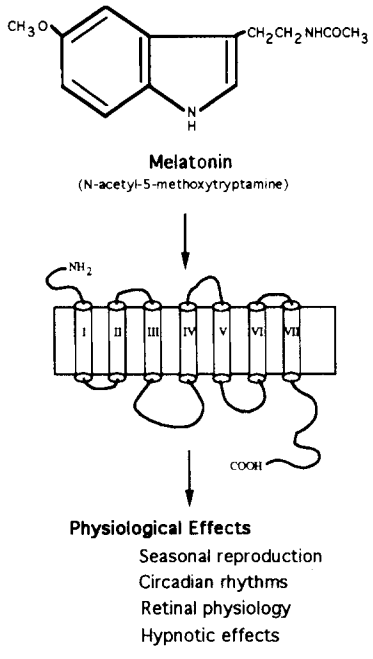

Figure 1. Melatonin Acts through High Affinity G Protein–Coupled Receptors to Elicit Its Physiological Effects

in day length in seasonally breeding mammals (reviewed by Arendt, 1995). These so-called photoperiodic responses are dramatic and include changes in reproductive status, body weight, coat color, and behavior. By providing a measure of night length and thereby day length, the nocturnal secretory pattern of melatonin regulates these seasonal responses.

Reproduction in humans may also be influenced by season, but the effect is quite subtle (reviewed by Arendt, 1995). Notably, preliminary reports have surfaced proclaiming success in using a melatonin–progestin combination as a contraceptive agent in humans. However, massive amounts of melatonin (75–300 mg) are given in these preparations, leading to pharmacological effects of the hormone on other hormone regulatory systems (e.g., direct actions on estrogen receptors). This potential for interaction with other systems should raise concern about the indiscriminate, unregulated use of melatonin in humans.

Melatonin can entrain (set the timing of) mammalian circadian rhythms and appears to function in concert with light to hold circadian rhythms in phase with prevailing environmental conditions (Reppert et al., 1994, and references therein). Importantly, the entraining effect of melatonin on the biological clock is weak compared with the potent entraining effect of light. In a natural setting, entrainment by melatonin may be most important during early development when retina-mediated light information cannot be processed (Davis and Mannion, 1988). In fact, during fetal life, at a time when the retina–SCN pathway has not yet formed, melatonin produced by the mother provides the developing SCN with entraining information. This maternal–fetal communication keeps the fetal clock entrained and in tune with the outside world until retina-mediated entrainment becomes functional during postnatal life.

Sound therapeutic applications of melatonin have been developed around its circadian effects (Arendt, 1995). Melatonin administered orally to humans has been used successfully to treat jet lag and some circadian-based sleep disorders. A critical feature of the circadian effect of melatonin is time of administration. Melatonin can alter circadian rhythms only during a restricted portion of the circadian cycle.

Melatonin also has potent biological effects on the vertebrate retina (reviewed by Cahill and Besharse, 1995). Studies in Xenopus and chick reveal that melatonin is made rhythmically in the retina and acts locally to inhibit retinal dopamine release and to affect photopigment disc shedding and phagocytosis. Melatonin is also produced by the mammalian retina, and at physiological levels it inhibits Ca^{2+}-dependent dopamine release in rabbit retina. Thus, melatonin may have several important regulatory functions in mammalian retina.

The most recently described effect of melatonin for which there is good scientific evidence is its ability to induce sleep in humans. Importantly, the hypnotic effect occurs when very low levels of melatonin are administered (0.1–0.3 mg); daytime administration only increased serum melatonin levels to the normal nocturnal range (Dollins et al., 1994). This hypnotic effect appears to be separable from melatonin's effect on circadian rhythms and may occur through a thermoregulatory mechanism (see references in Dollins et al., 1994).

The therapeutic potential of the hypnotic effects of melatonin is substantial. However, because of the time-sensitive activities of this hormone, clinical trials are clearly needed to identify the correct patient population and treatment regimens to optimize melatonin treatment. Unfortunately, melatonin's hypnotic capability has also been embellished, serving as a catalyst in initiating the current media blitz.

Melatonin Receptors Exist

Over the past 8 years, radioreceptor assays and quantitative in vitro autoradiography using the potent melatonin agonist, 2-[^{125}I]iodomelatonin ([^{125}I]Mel), have identified high affinity melatonin receptors in a variety of vertebrate species, including humans (reviewed by Dubocovich, 1995; see references in Reppert et al., 1994). These receptors have an equilibrium dissociation constant (K_D) of <100 pM. Their pharmacological specificity (rank order of inhibition of specific [^{125}I]Mel binding) is 2-iodomelatonin > melatonin > 6-chloromelatonin > 6-hydroxymelatonin > N-acetyl-5-hydroxytryptamine >> 5-HT. Receptor affinity is sensitive to guanine nucleotides, and activation of these receptors consistently leads to inhibition of adenylyl cyclase through a pertussis toxin–sensitive mechanism. High affinity melatonin receptors thus appear to belong to the superfamily of guanine nucleotide–binding protein (G protein)–coupled receptors.

In vitro autoradiography has localized high affinity melatonin receptors within individual brain nuclei and in a few nonneural sites. Significantly, these receptor sites appear to be the sites through which melatonin elicits its biological effects (reviewed by Dubocovich, 1995; Reppert et al., 1994). In most mammals, high affinity melatonin receptors are found in the SCN; SCN receptors probably mediate the circadian effects of melatonin. Receptors in the mediobasal hypothalamus of several photoperiodic rodents appear to be involved in melatonin's control of reproduction (Maywood and Hastings, 1995). Receptors in the inner plexiform layer of the retina may mediate melatonin's effects on retinal physiology. Melatonin receptors have been identified in the preoptic area, cerebral cortex, and thalamus of some mammals, which could mediate the hypnotic effects of the hormone.

Nonneural sites containing high affinity melatonin receptors include the hypophyseal pars tuberalis, a site involved in the photoperiodic regulation of prolactin and perhaps in other photoperiodic responses (Lincoln and Clarke, 1994). High affinity melatonin receptors have also been identified in cerebral and caudal arteries, which may be involved in thermoregulatory function in rodents (see references in Dubocovich, 1995).

A Family of Melatonin Receptors Is Cloned

A major advance in understanding melatonin's actions has been the recent cloning of a family of G protein–coupled receptors for melatonin. The strategy that succeeded in isolating the first melatonin receptor cDNA was expression

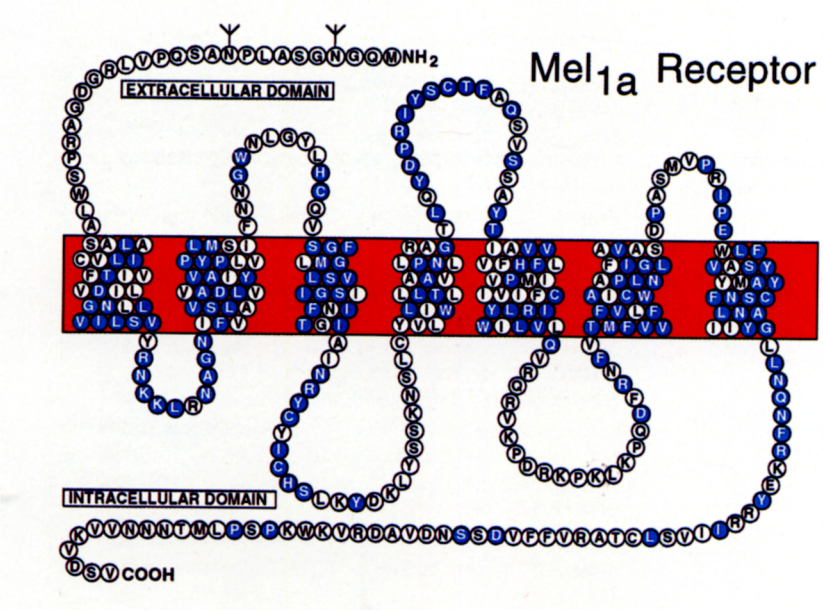

Figure 2. Membrane Topology of the Human Mel_{1a} Melatonin Receptor

Blue denotes amino acids identical among all available cDNAs, including representatives of each of the three receptor subtypes.

cloning (Ebisawa et al., 1994). The tissue source used for expression cloning took advantage of melatonin's ability to cause melanin aggregation in dermal melanophores of amphibians. This action of melatonin is mediated by a high affinity receptor that is coupled to inhibitory G_i. Thus, using a cDNA library constructed from an immortalized cell line of Xenopus laevis dermal melanophores and a mammalian cell expression cloning strategy, a high affinity melatonin receptor was cloned. The expressed recombinant Xenopus receptor exhibits pharmacological and functional characteristics that are indistinguishable from those of endogenous receptors in amphibian dermal melanophores (Ebisawa et al., 1994).

Structurally, the Xenopus receptor and related subtypes (see below) define a distinct receptor family within the large superfamily of G protein–coupled receptors (Figure 2). The melatonin receptor proteins are not similar in identity to any one particular group of G protein–coupled receptors, but are similar to a wide range of receptors within the G protein–coupled receptor superfamily (amino acid identity, ~25%). Distinguishing features of the melatonin receptor group include a NRY (rather than DRY or ERY) motif just downstream of the third transmembrane domain, a C(C/Y)ICHS motif immediately downstream of NRY, and a NAXXY (rather than NPXXY) motif in transmembrane domain 7 (Figure 2).

Using a polymerase chain reaction (PCR) approach based on the frog sequence, a high affinity melatonin receptor that was 60% identical at the amino acid level with the frog receptor was subsequently cloned from several mammals, including humans (Reppert et al., 1994). These mammalian receptors show >80% amino acid identity with each other and thus appear to be species homologs of the same receptor, designated the Mel_{1a} melatonin receptor. The expressed recombinant mammalian receptors exhibit pharmacological and functional characteristics (e.g., coupling to G_i) that are similar to their endogenous counterparts. The Mel_{1a} receptor gene is expressed in the rodent SCN and pars tuberalis, prominent sites of [^{125}I]Mel binding and the presumed sites of the circadian and some of the reproductive actions of melatonin, respectively. The Mel_{1a} melatonin receptor may account for all the [^{125}I]Mel binding observed by in vitro autoradiography in mammals. However, extremely low levels of receptor mRNA expression have precluded a firm determination as to whether the Mel_{1a} receptor is responsible for [^{125}I]Mel binding in some sites, such as blood vessels. The available evidence suggests that the Mel_{1a} receptor is the receptor through which melatonin exerts its circadian and reproductive actions in mammals.

A second melatonin receptor, designated the Mel_{1b} receptor, was recently cloned from humans (Reppert et al., 1995a; Table 1). The human Mel_{1b} receptor is 60% identical at the amino acid level to the human Mel_{1a} receptor. The expressed Mel_{1b} receptor exhibits ligand binding characteristics that are very similar to those of the Mel_{1a} receptor. The Mel_{1b} receptor is also coupled to inhibition of adenylyl cyclase. Mel_{1b} receptor mRNA is not detectable by in situ hybridization in the rat brain or pituitary, but reverse transcription (RT)–PCR shows that the Mel_{1b} receptor transcript is expressed in human retina. The Mel_{1b} receptor may mediate the reported actions of melatonin in the mammalian retina.

The Mel_{1a} and Mel_{1b} receptors are 60% identical at the amino acid level to each other, and each mammalian subtype is 60% identical to the Xenopus receptor. Thus, it seemed possible that the frog receptor represents a third subtype. Indeed, molecular cloning studies in chickens identified a high affinity melatonin receptor that is 80% identical at the amino acid level with the frog receptor, but only 60% identical with the Mel_{1a} and Mel_{1b} receptors (Reppert et al., 1995b). This subtype, designated the Mel_{1c} receptor, has pharmacological and functional properties similar to the mammalian Mel_{1a} and Mel_{1b} receptors (Table

Table 1. Characteristics of the G Protein–Coupled Melatonin Receptor Family

Melatonin Receptor Subtypes	K_D (pM)	Pharmacology	cAMP
Mel_{1a}	20–40	I > M ≥ 6 > N >> 5-HT	Inhibit
Mel_{1b}	160	I > M = 6 > N >> 5-HT	Inhibit
Mel_{1c}	20–60	I > M > 6 > N >> 5-HT	Inhibit

K_D determined using [^{125}I]Mel as the ligand. Abbreviations: I, 2-iodomelatonin; M, melatonin; 6, 6-hydromelatonin; N, N-acetyl-5-hydroxytryptamine; 5-HT, 5-hydroxytryptamine.

1). PCR analysis of additional melatonin receptor fragments from Xenopus and zebrafish provided further evidence of three distinct melatonin receptor subtypes in vertebrates (Reppert et al., 1995b). A Mel_{1c} receptor has not yet been cloned in mammals. It is possible that mammals evolved without a Mel_{1c} receptor gene or that sequence divergence is too great for detection by PCR and library screening. This issue can only be clarified by further molecular cloning studies.

The three melatonin receptor subtypes have similar gene structure (Reppert et al., 1994, 1995a, 1995b). The portions of the genes that encode the receptor proteins are composed of two exons, separated by large (>8 kb) introns. The conserved position of the intron splice site in the DNA encoding the first cytoplasmic loop of all three receptors suggests that alternatively spliced forms may exist. The two human melatonin receptor genes reside on different chromosomes (Mel_{1a} on 4q35.1 and Mel_{1b} on 11q21-22).

Demystification of Melatonin

Melatonin is a hormone. It has substantial biological effects and signals through a family of G protein–coupled receptors. The therapeutic potential of this hormone is in the early stages of evaluation, with the most promising areas exploiting its circadian and hypnotic effects.

Delineating melatonin receptor structure provides important new tools for investigating fundamental mechanisms of melatonin action. For example, gene targeting can now be performed to determine the precise physiological role(s) of each receptor subtype. Elucidating melatonin receptor gene structure may also allow a molecular means of accessing the biological clock in the SCN (e.g., targeted gene delivery). With recombinant melatonin receptors in hand, it should now be possible to identify highly specific agonists and antagonists that may be of therapeutic use in treating jet lag and circadian-based sleep disorders and of agricultural value in modulating the timing of reproduction in seasonally breeding species.

René Descartes, the French philosopher and mathematician, proclaimed in the 17th century that the pineal gland was the "seat of the soul." Even Descartes would be astonished at the properties currently being attributed to melatonin. Continued progress in understanding the cellular and molecular actions of melatonin will hopefully remove the mystery surrounding this hormone. The cure for melatonin madness is to ignore the hyperbole and histrionics and focus instead on hypothesis testing and sound science.

Selected Reading

Arendt, J. (1995). Melatonin and the Mammalian Pineal Gland (London: Chapman and Hill).

Becker-Andre, M., Wiesenberg, I., Schaeren-Wiemers, N., Andre, E., Missbach, M., Saurat, J.-H., and Carlberg, C. (1994). J. Biol. Chem. *269*, 28531–28534.

Cahill, G.M., and Besharse, J.C. (1995). Retinal Eye Res. *14*, 267–291.

Coon, S.L., Rosenboom, P.H., Baler, R., Weller, J.L., Namboodiri, M.A.A., Koonin, E.V., and Klein, D.C. (1995). Science *270*, 1681–1683.

Davis, F.C., and Mannion, J. (1988). Am. J. Physiol. *255*, R439–R448.

Dollins, A.B., Zhdanova, I.V., Wurtman, R.J., Lynch, H.J., and Deng, M.H. (1994). Proc. Natl. Acad. Sci. USA *91*, 1824–1828.

Dubocovich, M.L. (1995). Trends Pharmacol. Sci. *16*, 50–56.

Ebisawa, T., Karne, S., Lerner, M.R., and Reppert, S.M. (1994). Proc. Natl. Acad. Sci. USA *91*, 6133–6137.

Goto, J., Oshima, I., Hasegawa, M., and Ebihara, S. (1994). Mol. Brain Res. *21*, 349–354.

Lincoln, G.A., and Clarke, I.J. (1994). J. Neuroendocrinol. *6*, 251–260.

Maywood, E.S., and Hastings, M.H. (1995). Endocrinology *136*, 144–153.

Pierpaoli, W., and Regelson, W. (1994). Proc. Natl. Acad. Sci. USA *91*, 787–791.

Pierpaoli, W., and Regelson, W. (1995). The Melatonin Miracle (New York: Simon and Schuster).

Reiter, R.J. (1995). FASEB J. *9*, 526–533.

Reiter, R.J., and Robinson, J. (1995). Melatonin (New York: Bantam Books).

Reppert, S.M., Weaver, D.R., and Ebisawa, T. (1994). Neuron *13*, 1177–1185.

Reppert, S.M., Godson, C., Mahle, C.D., Weaver, D.R., Slaugenhaupt, S.A., and Gusella, J.F. (1995a). Proc. Natl. Acad. Sci. USA *92*, 8734–8738.

Reppert, S.M., Weaver, D.R., Cassone, V.M., Godson, C., and Kolakowski, L.F., Jr. (1995b). Neuron *15*, 1003–1015.

Wiesenberg, I., Missbach, J., Kahlen, J.P., Schrader, M., and Carlberg, C. (1995). Nucl. Acids Res. *23*, 327–333.