

MELATONIN RECEPTORS



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Introduction

Melatonin (5-methoxy-*N*-acetyltryptamine) is a hormone which was first isolated from the bovine pineal gland in 1958.¹ The synthesis of melatonin takes place primarily in the pineal gland, via a two step process; *N*-acetylation of serotonin by arylalkylamine *N*-acetyltransferase (AA-NAT, EC 2.3.1.87) to give *N*-acetylserotonin, followed by methylation of the 5-hydroxy group by hydroxyindole-O-methyltransferase (HIOMT, EC 2.1.1.4) to yield melatonin. Characteristically, pineal melatonin is synthesised and secreted in a circadian manner with high levels occurring in all species at night. In mammals, the melatonin rhythm is generated by an endogenous circadian clock in the suprachiasmatic nucleus (SCN) of the hypothalamus, which is entrained by the light/dark cycle to the 24h day.

Melatonin regulates a number of neuroendocrine and physiological processes. Seasonal changes in various aspects of physiology in photoperiodic species, such as sheep and hamsters, are controlled by actions of melatonin in the hypothalamus and the pars tuberalis of the pituitary. Melatonin administration can also entrain the circadian clock by a direct action on the SCN. This response has led to considerable interest in its potential in treating disordered circadian rhythms which occur in jet-lag, shift-work, some blind subjects and in delayed/advanced sleep phase syndromes.² Melatonin also inhibits dopamine release from amacrine cells within the retina,³ and can enhance vasoconstriction in the rat tail artery.⁴ Melatonin also has a well-established hypnotic action,⁵ and

has been considered to have a role in sleep initiation as the trigger for opening the circadian-dependent "sleep-gate".⁶ Many studies have also indicated an influence on immune function⁷ and antioxidant actions.⁸

MT₁ and MT₂ Receptors

The development of 2-[¹²⁵I]iodomelatonin, a high-affinity melatonin receptor agonist, as a radioligand has allowed the distribution and pharmacological characteristics of melatonin receptors to be examined in various central and peripheral tissues in a number of species.^{9, 10} Putative melatonin receptors were initially classified into two types, ML₁ and ML₂, based on pharmacological and kinetic differences in 2-[¹²⁵I]iodomelatonin binding.¹¹ Binding sites in mammalian retina and pars tuberalis (ML₁, 2-iodomelatonin>melatonin>>*N*-acetylserotonin) have high affinity and a pharmacology which corresponds closely to that of the functional melatonin receptor characterised in rabbit retina and rat tail artery.^{11,12} In contrast, 2-[¹²⁵I]iodomelatonin binding to hamster brain membranes is low affinity with a quite different pharmacology (ML₂, melatonin=*N*-acetylserotonin).^{2,11} Two mammalian ML₁ subtypes have subsequently been cloned.^{13, 14} The classification of melatonin receptors approved by the nomenclature committee of IUPHAR¹⁵ now designates these as MT₁, which corresponds to the subtype previously known as ML_{1A} or Mel_{1a}, and MT₂, corresponding to the subtype previously known as ML_{1B} or Mel_{1b}. MT₁ and MT₂ melatonin receptors are members of the superfamily of putative seven transmembrane domain G-protein coupled receptors.

Recombinant MT₁ receptors are coupled to adenylate cyclase inhibition¹³ and possibly to phosphatidylinositol hydrolysis, via a pertussis toxin sensitive G-protein.¹⁶ MT₁ receptor mRNA has been detected in the SCN, pars tuberalis and other parts of the brain including the hypothalamus, cerebellum and cerebral cortex.^{17, 18} In the rat caudal artery, the melatonin receptor enhancing electrically-evoked contraction has been pharmacologically characterised as the MT₁ receptor subtype.¹⁹ Like the MT₁ subtype, activation of recombinant MT₂ receptors also inhibits cyclic AMP synthesis.¹⁴ Melatonin

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inhibition of dopamine release from the retina is mediated by the MT_2 receptor subtype.²⁰ Pharmacological studies suggest that the phase shifting effect of melatonin on circadian rhythms may also be mediated by the MT_2 receptor subtype,²¹ although other experiments using an MT_1 receptor knockout mouse²² indicate that this receptor is responsible for the acute inhibition of SCN firing by melatonin.

Pharmacology of MT_1 and MT_2 Receptors

A number of studies have described putative melatonin receptor agonists and antagonists (for reviews see²³⁻²⁶), and much has been learnt about how melatonin binds to and activates its receptors. The 5-methoxyl group is important for high affinity binding.²⁷ *N*-acetyl on the C-3 side-chain is not optimal and replacement with propanoyl or butanoyl increases binding affinity and potency of agonists,²⁷ although this may not be the case for antagonists.²⁸ 2-Iodo-*N*-butanoyl-5-methoxytryptamine (2-IbMT) ($K_i = 15$ pM in binding assays) is a very potent melatonin receptor agonist in a functional assay on *Xenopus laevis* melanophores ($IC_{50} = 6$ pM).²⁹ Confirmationally restricted indole and non-indole analogues have established the active conformation of the 3-ethanamide side-chain.^{30,31} Substitutions at the 2-position of the indole ring of melatonin increase affinity and potency considerably; in part because steric effects restrict the flexible C-3 side-chain allowing easier docking at the active site of the receptor. For example, 2-position substitution improves affinity at both receptor subtypes;²⁷ 2-iodomelatonin and 2-phenylmelatonin show a ~ 10-fold improvement in affinity ($K_i \sim 60$ pM) over melatonin itself. Other analogues which have been used to characterise melatonin binding sites are 6-chloromelatonin, an agonist²⁰ and *N*-acetyltryptamine, a partial agonist.²⁷ Radioligand binding assays on recombinant melatonin receptor subtypes indicate that the MT_2 receptor has less stringent requirements at the 5-position and will tolerate substituents which lead to reductions in affinity at the MT_1 subtype.²⁷ For example, 5-benzyloxy *N*-acetyltryptamine is an agonist with 18-fold selectivity for the MT_2 subtype. Another compound, KI17 has 3-fold higher affinity for the MT_2 subtype than melatonin, but 28-fold lower affinity at the MT_1 subtype. This compound is a selective MT_2 receptor agonist (90-fold selective).³²

Relatively few melatonin receptor antagonists have been reported. Luzindole was the first competitive receptor antagonist described.³³ It has slight selectivity for the MT_2 subtype (18-fold), but a congener, DH97, is much more selective (90-fold) and has higher affinity.²⁸ Other MT_2 selective antagonists have been described: these include 4-P-PDOT and related tetralines²⁰ and K185.³⁴ GR128107²⁰ was initially described as a melatonin receptor antagonist but subsequent work showed that it acted as a partial agonist at recombinant MT_1 and MT_2 receptors and in

Xenopus melanophores.³⁵ No MT_1 selective agonists or antagonists have yet been discovered.

MT_3 Receptors

The ML_2 2-[¹²⁵I]iodomelatonin binding site, initially shown to be widely distributed in hamster brain,¹¹ has also been found in peripheral tissues³⁶ and RPMI hamster melanoma cells³⁷ using a selective radioligand, 2-[¹²⁵I]iodo-5-methoxycarbonylamino-*N*-acetyltryptamine (GR135531). The correct IUPHAR designation for this site is now MT_3 . In RPMI cells, activation of MT_3 sites increases phosphatidylinositol turnover.³⁷ Prazosin is an antagonist at this site. The MT_3 site has not yet been cloned, no selective antagonists are available, nor has it been linked with a specific tissue function.

Future Trends

A cDNA encoding a putative G-protein coupled receptor homologous to the cloned MT_1/MT_2 receptors has been isolated from human pituitary. When expressed in COS-1 cells, this melatonin related receptor (MRR) does not bind [³H]melatonin or 2-[¹²⁵I]iodomelatonin, but the MRR shares structural motifs and gene structure with the melatonin receptor and has a similar tissue distribution.³⁸ The natural ligand for MRR is not known.

Selective MT_1 melatonin receptor subtype agonists and antagonists are needed, but attempts to discover selective MT_1 ligands have not yet borne fruit. The development of selective MT_2 receptor agonists and antagonists will continue. The availability of such selective melatonin receptor ligands will lead to a better understanding of the physiological and pathophysiological role(s) of melatonin in animals and man, and will be useful in defining the cellular mechanisms of action of this hormone. In the future, analogues of melatonin may be of value in treating sleep and circadian rhythm disturbances and may also have other therapeutic applications.

References

1. **Lerner et al** (1958) Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J.Am.Chem.Soc.* **80**2587.
2. **Hagan and Oakley** (1995) Melatonin comes of age? *TIPS* **16**81.
3. **Dubocovich** (1985) Characterisation of a retinal melatonin receptor. *J.Pharmacol.Exp.Ther.* **234** 395.
4. **Krause et al** (1995) Melatonin receptors mediate potentiation of contractile responses to adrenergic nerve stimulation in the rat caudal artery. *Eur.J.Pharmacol.* **276**207.
5. **Tzischinsky and Lavie** (1995) Melatonin possesses time-dependent hypnotic effect. *Sleep* **17**638.
6. **Krauchi et al** (2000) Functional link between distal vasodilatation and sleep onset latency? *Am.J.Physiol. (Regulatory Integrative Comp. Physiol.)* **278**R741.
7. **Liebmann et al** (1997) Melatonin and the immune system. *Int.Arch.Allergy Appl.Immunol.* **112**203.
8. **Reiter et al** (1999) Melatonin and tryptophan derivatives as free radical scavengers and antioxidants. *Adv.Exp.Med.Biol.* **467**379.
9. **Morgan et al** (1994) Melatonin receptors: Localization, molecular pharmacology and physiological significance. *Neurochem.Int.* **24** 101.
10. **Vanecek** (1999) Cellular mechanisms of melatonin action. *Phys.Rev.* **78**687.
11. **Dubocovich** (1988) Pharmacology and function of melatonin receptors. *FASEB J.* **2**2765.
12. **Ting et al** (1997) Studies on the vasoconstrictor action of melatonin and putative melatonin receptor ligands in the tail artery of juvenile Wistar rats. *Br.J.Pharmacol.* **122**1299.
13. **Reppert et al** (1994) Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. *Neuron* **13** 1177.
14. **Reppert et al** (1995) Molecular characterization of a second melatonin receptor expressed in human retina and brain; the Mel_{1b} melatonin receptor. *Proc.Natl.Acad.Sci.USA* **92**8734.
15. **Dubocovich et al** (1998) Melatonin receptors. The IUPHAR Compendium of Receptor Characterization and Classification, pp87-193, IUPHAR Media, London, UK.
16. **Godson and Reppert** (1997) The Mel_{1a} melatonin receptor is coupled to parallel signal transduction pathways. *Endocrinology* **138**397.
17. **Mazzucchelli et al** (1996) The melatonin receptor in the human brain: cloning experiments and distribution studies. *Brain Res.Mol.Brain Res.* **39** 117.
18. **Bittman and Weaver** (1990) The distribution of melatonin binding sites in neuroendocrine tissue of the ewe. *Biol.Reprod.* **43**986.
19. **Ting et al** (1999) Molecular and pharmacological evidence for MT₁ melatonin receptor subtype in the tail artery of juvenile Wistar rats. *Br.J.Pharmacol.* **127**987.
20. **Dubocovich et al** (1997) Melatonin receptor antagonists that differentiate between the human Mel_{1a} and Mel_{1b} recombinant subtypes are used to assess the pharmacological profile of the rabbit retina ML₁ presynaptic heteroreceptor. *Naunyn-Schmied.Arch.Pharmacol.* **355**365.
21. **Dubocovich et al** (1998) Selective MT₂ melatonin receptor antagonists block melatonin-mediated phase advances of circadian rhythms. *FASEB J.* **12** 1211.
22. **Liu et al** (1997) Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. *Neuron* **19**91.
23. **Mahle et al** (1997) Melatonin receptor ligands and their potential clinical applications. *Ann.Rep.Med.Chem. Ch.* **4** pp31-40.
24. **Witt-Enderby and Li** (2000) Melatonin receptors and ligands. *Vitam.Horm.* **58**321.
25. **Steinhilber and Carlberg** (1999) Melatonin receptor ligands. *Exp.Opin.Ther.Patents* **9**281.
26. **Spadoni et al** (1999) Structure-affinity relationships of indole-based melatonin analogs. *Biol.Signals Recept.* **8** 15.
27. **Sugden et al** (1997) Melatonin receptor pharmacology: toward subtype specificity. *Biol.Cell* **89**531.
28. **Teh and Sugden** (1998) Comparison of the structure-activity relationships of melatonin receptor agonists and antagonists: lengthening of the *N*-acyl side-chain has differing effect on potency on *Xenopus* melanophores. *Naunyn-Schmied.Arch.Pharmacol.* **358**522.
29. **Sugden and Rowe** (1994) 2-Iodo-*N*-butanoyl-5-methoxytryptamine: a potent melatonin receptor agonist. *Pharmacol.Comm.* **4**267.
30. **Davies et al** (1998) Mapping the melatonin receptor. 5. Melatonin agonists and antagonists derived from tetrahydrocyclopent[b]indoles, tetrahydrocarbazoles and hexahydrocyclohept[b]indoles. Differences in binding and biological activity between enantiomers. *J.Med.Chem.* **41** 451.
31. **Mathé-Allainmat et al** (1996) Synthesis of 2-amido-2,3-dihydro-1H-phenalene derivatives as new conformationally restricted ligands for melatonin receptors. *J.Med.Chem.* **39**3089.
32. **Sugden et al** (1999) Design of subtype selective melatonin receptor agonists and antagonists. *Reprod.Nutr.Develop.* **39**335.
33. **Dubocovich** (1988) Luzindole (N-0774): A novel melatonin receptor antagonist. *J.Pharmacol. Exp.Ther.* **246**902.
34. **Faust et al** (2000) Mapping the melatonin receptor. 6. Melatonin agonists and antagonists derived from 6H-isoindolo[2,1-a]indoles, 5,6-dihydroindolo[2,1-a]isoquinolines and 6,7-dihydro-5H-benzo[3,4]azepino[1,2-a]indoles. *J.Med.Chem.* **43** 1050.
35. **Teh and Sugden** (1999) The putative melatonin receptor antagonist GR128107 is a partial agonist on *Xenopus laevis* melanophores. *Br.J.Pharmacol.* **126**1237.
36. **Molinari et al** (1996) 2-[¹²⁵I]Iodo-5-methoxycarbonylamino-*N*-acetyltryptamine; a selective radioligand for the characterisation of melatonin ML₂ binding sites. *Eur.J.Pharmacol.* **301** 159.
37. **Eison and Mullins** (1993) Melatonin binding sites are functionally coupled to phosphoinositide hydrolysis in Syrian hamster RPMI 1846 hamster melanoma cells. *Life Sci.* **53**393.
38. **Reppert et al** (1996) Cloning of a melatonin-related receptor from human pituitary. *FEBS Lett.* **386**219.

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0357	N-Acetyltryptamine	Melatonin partial agonist (MT ₁ / MT ₂). Also MT ₃ antagonist
0443	6-Chloromelatonin	Melatonin agonist
1218	DH 97	MT ₂ receptor antagonist
0896	GR-135,531	High affinity melatonin MT ₃ ligand
0737	2-Iodomelatonin	High affinity melatonin agonist
0765	2-Iodo-N-butanoyl-5-methoxytryptamine	Potent, high affinity melatonin MT ₁ / MT ₂ agonist
0877	Luzindole	Competitive melatonin MT ₁ / MT ₂ antagonist
0766	5-Methoxy-N-cyclopropanoyltryptamine	Melatonin agonist
1035	8-M-PDOT	Melatonin agonist
1034	4-P-PDOT	MT ₂ antagonist
0680	2-Phenylmelatonin	Melatonin agonist
0623	Prazosin	MT ₃ antagonist, also α_1 antagonist

Melatonin Receptors, Tocris Reviews No. 14, June 2000

Published and distributed by Tocris Cookson

Editor: Samantha Manley, Ph.D.

Managing Editor: Duncan Crawford, Ph.D.

Design and Production: Jane Champness; Lacia Ashman, MA

ML(0600)

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