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 function[7] and antioxidant actions.[8]

MT_1 and MT_2 Receptors

The development of 2-[^{125}I]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, ML_1 and ML_2, based on pharmacological and kinetic differences in 2-[^{125}I]iodomelatonin binding.[11] Binding sites in mammalian retina and pars tuberalis (ML_1, 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-[^{125}I]iodomelatonin binding to hamster brain membranes is low affinity with a quite different pharmacology (ML_2, melatonin=*N*-acetylserotonin).[2,11] Two mammalian ML_1 subtypes have subsequently been cloned.[13, 14] The classification of melatonin receptors approved by the nomenclature committee of IUPHAR[15] now designates these as MT_1, which corresponds to the subtype previously known as ML_{1A} or Mel_{1a}, and MT_2, corresponding to the subtype previously known as ML_{1B} or Mel_{1b}. MT_1 and MT_2 melatonin receptors are members of the superfamily of putative seven transmembrane domain G-protein coupled receptors.

Recombinant MT_1 receptors are coupled to adenylate cyclase inhibition[13] and possibly to phosphatidylinositide hydrolysis, via a pertussis toxin sensitive G-protein.[16] MT_1 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 MT_1 receptor subtype.[19] Like the MT_1 subtype, activation of recombinant MT_2 receptors also inhibits cyclic AMP synthesis.[14] Melatonin

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 mouse[22] 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 see[23-26]), 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 ~ 60 pM) over melatonin itself. Other analogues which have been used to characterise melatonin binding sites are 6-chloromelatonin, an agonist[20] 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, KII7 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 tetralines[20] and K185.[34] GR128107[20] 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-[^{125}I]iodomelatonin binding site, initially shown to be widely distributed in hamster brain,[11] has also been found in peripheral tissues[36] and RPMI hamster melanoma cells[37] using a selective radioligand, 2-[^{125}I]iodo-5-methoxycarbonylamino-*N*-acetyltryptamine (GR135531). The correct IUPHAR designation for this site is now *MT_3*. In RMPI 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 [^{3}H]melatonin or 2-[^{125}I]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.

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MELATONIN LIGANDS AVAILABLE FROM TOCRIS

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

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