Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 66 (2003) 315–320
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Mutagenesis studies of the human MT_2 melatonin receptor

Matthew J. Gerdin[a,c,d,1], Faika Mseeh[a,d,1,2], Margarita L. Dubocovich[a,b,c,d,*]

[a]*Department of Molecular Pharmacology and Biological Chemistry (S215), The Feinberg School of Medicine, Northwestern University, 303 East Chicago Avenue, Chicago, IL 60611-3008, USA*
[b]*Department of Psychiatry and Behavioral Sciences, The Feinberg School of Medicine, Northwestern University, Chicago, IL 60611-3008, USA*
[c]*Northwestern University Institute for Neuroscience, Northwestern University, Chicago, IL 60611-3008, USA*
[d]*Northwestern Drug Discovery Program, Northwestern University, Chicago, IL 60611-3008, USA*

Received 23 December 2002; accepted 3 March 2003

Abstract

Melatonin mediates its physiological effects through activation of high affinity G protein-coupled receptors. The vertebrate MT_1, MT_2 and Mel_{1c} melatonin receptors are molecularly and pharmacologically distinct. Three molecular models of melatonin recognition for the MT_1 and/or Mel_{1c} melatonin receptors have been proposed. To determine if these models applied to the MT_2 melatonin receptor, we mutated seven conserved residues to alanine in the hMT_2 melatonin receptor and expressed the receptors in HEK-293 cells. Competition of melatonin for 2-[^{125}I]-iodomelatonin binding revealed that mutation of Asn 16 in TM4 or His 7 in TM5 of the hMT_2 melatonin receptor significantly decreased the binding affinity for melatonin when compared with wild-type. In addition, competition of 4P-ADOT, *N*-acetyltryptamine, luzindole, and 5-methoxytryptophol for 2-[^{125}I]-iodomelatonin binding suggested Asn 16 in TM4 may facilitate binding of the 5-methoxy group of the melatonin molecule to the hMT_2 melatonin receptor. Trp 13 or Phe 6 in TM6 while not critical for melatonin binding, may interact with aromatic regions of luzindole and 4P-ADOT. Mutation of Ser 8 or Ser 12 in TM3, or Ser 6 in TM7 did not affect the affinity of melatonin for competition with 2-[^{125}I]-iodomelatonin to the hMT_2 melatonin receptor, although equivalent serines (Ser 8 and Ser 12 in TM3) were reported to be critical for melatonin binding to the hMT_1 melatonin receptor. Thus these results are the first to identify residues within the transmembrane regions of the hMT_2 melatonin receptor critical for melatonin binding, highlighting potential structural differences between the MT_1 and MT_2 melatonin receptor binding pockets.

Keywords: Melatonin; Site-directed mutagenesis; MT_1 melatonin receptor; MT_2 melatonin receptor; HEK cells; Ligand binding

1. Introduction

Melatonin, a hormone produced by the pineal gland and retina, is released following a circadian rhythm with high levels at night. It regulates visual, circadian, cardiovascular and neuroendocrine functions through activation of high-affinity cell membrane GPCRs [1,2]. The predominate signaling pathway for melatonin receptors is through inhibition of cAMP formation via a pertussis sensitive G-protein [2]. Three full-length melatonin receptors have been cloned: the mammalian MT_1 and MT_2 [3,4] and the non-mammalian Mel_{1c} cloned from *Xenopus* [5] and chick brain [6].

All GPCRs, including the melatonin receptors, share the presence of seven hydrophobic regions that are believed to form a bundle of α-helical TM domains connected by alternating intracellular and extracellular hydrophilic loops [7]. The actual arrangement and three-dimensional structure of most GPCRs are unknown; however, mutagenesis studies of several receptors revealed a similar organization of the GPCRs functional domains. It is believed that the TM domains contribute to the formation of the ligand-binding pocket, whereas the amino acids of the intracellular loops interact with G proteins [8].

In absence of direct structural data, most researchers have relied on a combination of site-directed mutagenesis studies and molecular modeling approaches to understand the structural basis of ligand binding. Current models of GPCR structure are basically derived from analogies with

* Corresponding author. Tel.: +1-312-503-8005; fax: +1-312-503-2334.
E-mail address: mdubo@northwestern.edu (M.L. Dubocovich).
[1] Authors contributed equally to this work.
[2] Present address: Lexicon Genetics Incorporated, 8800 Technology Forest Place, The Woodlands, TX 77381-1106, USA.
Abbreviations: GPCR, G protein-coupled receptor; TM, transmembrane; HEK, human embryonic kidney; WT, wild-type; h, human.

0006-2952/03/$ – see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0006-2952(03)00239-9

high-resolution crystal structures of bacteriorhodopsin (*Halobacterium salinarium*) [9] and bovine rhodopsin [10]. Three different models for melatonin recognition at its receptor have been proposed based on the amino acid sequences of two melatonin receptor types (MT_1 and Mel_{1c}), the helical structures of rhodopsin [11] and bacteriorhodopsin [12,13], and radioligand affinity constants.

Sugden *et al.* [12] have proposed a melatonin receptor binding pocket model based on the existing model for the serotonin receptor (5-HT_{2c}). The 5-HT_{2c} receptor binding domain was devised from site-directed mutagenesis studies and homology with the TM domains of bacteriorhodopsin. A putative melatonin receptor binding site was proposed using the molecular electrostatic potential map of melatonin and its similarity to serotonin. Modeling of the putative TM regions of the *Xenopus* melatonin receptor (Mel_{1c}), suggested that hydrogen bonding occurs between the 5-methoxy and the amide groups of melatonin with complimentary serine (Ser 8 in TM3) and asparagine (Asn 16 in TM4) residues, respectively. A tryptophan (Trp 13) in TM6 was suggested to overlap with the indole ring of melatonin allowing charge transfer between receptor and ligand. Likely interactions between three hydrophobic non-polar residues and the methoxymethyl group at the 5-position (Ile 21 in TM2), the amide methyl group (Val 19 in TM4), and methylene side-chain of melatonin (Ile 1 in TM5) were also postulated.

Grol and Jansen [13] proposed a model based on the primary amino acid sequence of the Mel_{1c} melatonin receptor and the helical structure of bacteriorhodopsin. In this model, the amide group of melatonin interacts with two serine residues (Ser 8 and Ser 12 in TM3), one being a hydrogen-bond donor and the other one a hydrogen-bond acceptor. A histidine residue (His 7 in TM5) forms a hydrogen-bond with the methoxy-O in melatonin. Additionally, possible stabilizing interactions may occur between the aromatic rings of the phenylalanines (Phe 17 in TM4 and Phe 8 in TM7) and the tryptophan (Trp 1 in TM7).

Navajas *et al.* [11] proposed a model for melatonin recognition at its receptor based on the primary structures of the MT_1 and Mel_{1c} melatonin receptors as well as the deduced secondary structure of rhodopsin. This model postulates that the 5-methoxy group of melatonin interacts with a fully conserved histidine in TM5. A fully conserved valine within the same TM provides the aliphatic milieu for the methyl group on the 5-oxygen. The *N*-acetyl group of melatonin interacts with a conserved serine (Ser 6 in TM7) allowing hydrogen bonding with the carbonyl oxygen. A fully conserved alanine (Ala 10) within the same TM could also be an anchor for the methyl group on the carbonyl group. Finally, an aromatic interaction between the indole ring and a phenylalanine (Phe 9 in TM6) was suggested.

Recently, aspects of these models have been validated using the MT_1 melatonin receptor [14,15]. In contrast, only two extracellular cysteines have been identified as critical for melatonin binding to the hMT_2 melatonin receptor [16], and nothing is known about amino acids within the TM helices involved in melatonin binding to the MT_2 melatonin receptor. To further evaluate these proposed molecular models for melatonin receptors and identify critical amino acids necessary for melatonin binding to the MT_2 melatonin receptor, we mutated several conserved amino acids and determined their radioligand affinity constants.

2. Materials and methods

2.1. Materials

cDNA containing the complete coding region of the hMT_2 receptor (human Mel_{1b}; cloned into pcDNA-3) was provided by Dr. S.M. Reppert (Department of Neurobiology, University of Massachusetts Medical School, Worcester, MA) [4]. The oligonucleotides used for site-directed mutagenesis and DNA sequencing were synthesized in the Biotechnology Facility of Northwestern University. Restriction enzymes were purchased from Promega. Effectene transfection and plasmid DNA purification kits were obtained from Qiagen. Cell culture products were obtained from Invitrogen. 2-[^{125}I]-Iodomelatonin (SA: 2000 Ci/mmol) was purchased from Amersham. Melatonin, 5-methoxytryptophol, and other general reagents were obtained from Sigma Chemical Co. *N*-Acetyltryptamine was donated by Eli Lilly and luzindole (2-benzyl-*N*-acetyltryptamine) was purchased from Tocris. 4-Phenyl-2-acetamidotetraline (4P-ADOT) was synthesized in the Department of Medical Chemistry at the University of Groningen, The Netherlands.

2.2. FLAG epitope tagging, site-directed mutagenesis, and transient transfection

Generation of the N-terminal FLAG hMT_2 melatonin receptor fusion protein, mutation of residues in the FLAG-tagged hMT_2 melatonin receptor using the PCR-based QuickChange site-directed mutagenesis kit (Stratagene), and transient transfection of HEK cells with these constructs using Effectene (Qiagen) have been previously described [16].

2.3. 2-[^{125}I]-Iodomelatonin binding

2-[^{125}I]-Iodomelatonin binding was determined in whole cell lysates (5–25 μg protein/tube) as previously described [16]. Saturation analyses were performed with 2-[^{125}I]-iodomelatonin (~10–4000 pM). Non-specific binding was determined in the presence of 1 μM melatonin. K_d and B_{max} values were calculated from saturation isotherms fitted by nonlinear regression analysis. The IC_{50} values were obtained from curves generated from competition of melatonin (1 pM to 1 μM), luzindole (10 pM to 10 μM), 4P-ADOT (1 pM to 1 μM), *N*-acetyltryptamine (100 pM to 100 μM), and 5-methoxytryptophol (100 pM to 100 μM)

for 2-[^{125}I]-iodomelatonin (~100 pM) binding. K_i values were calculated from IC_{50} values using the Cheng and Prusoff equation [17]. Analysis of ligand binding constants was performed using the Prism program (GraphPad Software Inc.). Statistical significance was determined by one-way ANOVA with Dunnett post-test. A value of $P < 0.05$ was taken as statistically significant.

3. Results and discussion

To identify critical amino acids necessary for melatonin binding to the hMT_2 melatonin receptor, seven conserved residues within the TM regions of the MT_1, MT_2, and Mel_{1c} melatonin receptors were mutated to alanine by site-directed mutagenesis: Ser 8 in TM3, Ser 12 in TM3, Asn 16 in TM4, His 7 in TM5, Phe 6 in TM6, Trp 13 in TM6, and Ser 6 in TM7 (Fig. 1). These residues were selected based on previous molecular models for melatonin binding to its receptor [11–13]. Saturation analysis of 2-[^{125}I]-iodomelatonin binding to HEK cells transiently transfected with either WT or mutant FLAG-tagged hMT_2 melatonin receptors revealed that mutation of His 7 in TM5 significantly decreased and mutation of Trp 13 in TM6 significantly increased the binding affinity (K_d) compared to WT (Table 1). Receptor density was significantly reduced by mutation of Trp 13 in TM6 or Ser 8 in TM3 compared to WT possibly suggesting alterations in receptor trafficking or membrane insertion (Table 1).

To validate predictions made by existing molecular melatonin receptor models, we determined binding affinities of WT and mutant FLAG-tagged hMT_2 melatonin receptors by competition of ligands of various chemical structures for 2-[^{125}I]-iodomelatonin binding. Four indole ligands and one amidotetraline ligand possessing different side chains were selected to assess key interactions between these molecules and the seven conserved residues within the hMT_2 melatonin receptor. Competition analysis revealed that only two of the mutated seven conserved residues (Asn 16 in TM4 and His 7 in TM5) reduced the

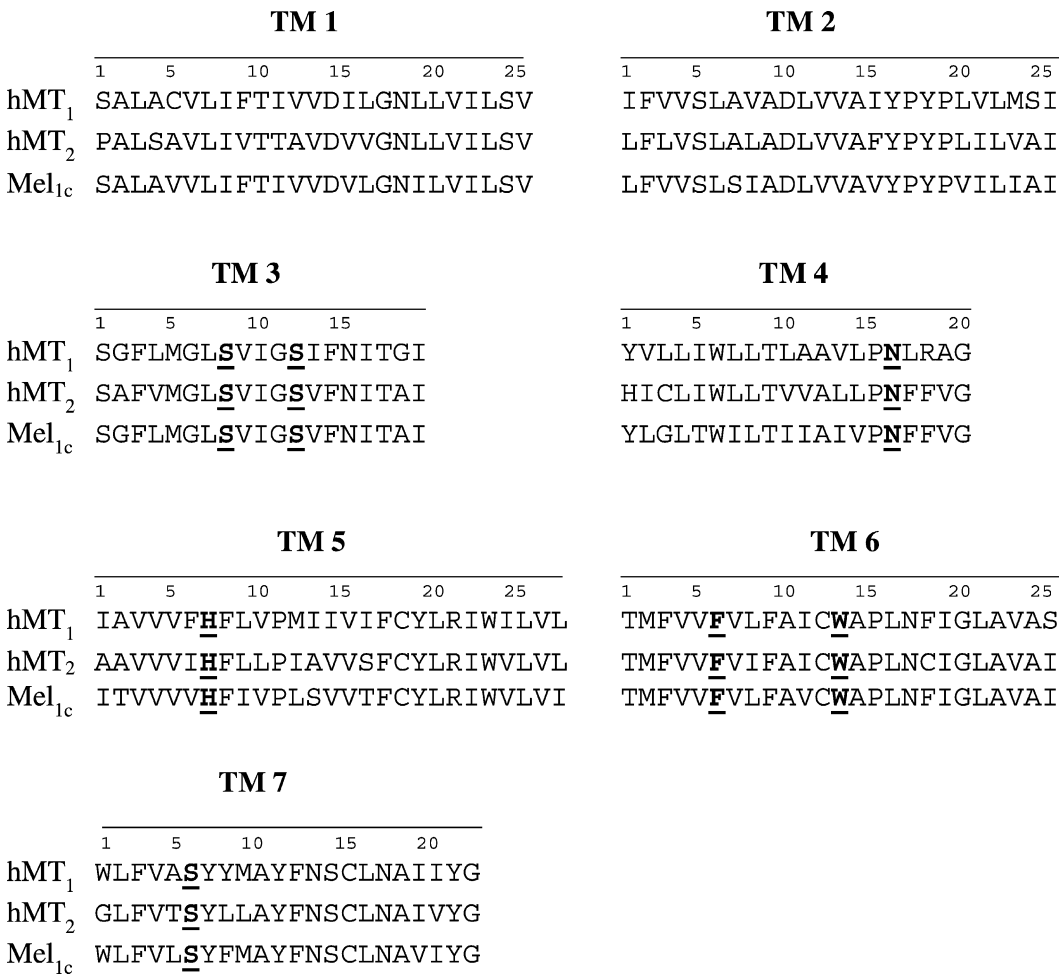

Fig. 1. Amino acid sequences of the putative seven transmembrane helices of the MT_1, MT_2, and *Xenopus* Mel_{1c} melatonin receptors. The amino acid residues are shown in one-letter code. The numbers indicate the position of each residue in the transmembrane domains. The underlined amino acids indicate the conserved residues mutated in the FLAG-tagged hMT_2 melatonin receptor and previously postulated [11–13] to be essential for melatonin binding: Ser 8 in TM3 (S123), Ser 12 in TM3 (S127), Asn 16 in TM4 (N175), His 7 in TM5 (H208), Phe 6 in TM6 (F257), Trp 13 in TM6 (W264), and Ser 6 in TM7 (S293).

Table 1
Affinity constants for the FLAG-tagged WT and mutant hMT_2 melatonin receptors

Receptor	2-[^{125}I]-Iodomelatonin binding						
	Saturation analysis		Competition analysis (K_i)				
	K_d (nM)	B_{max} (fmol/mg protein)	MLT (nM)	NAT (nM)	LUZ (nM)	4P-ADOT (nM)	5-MTP (μM)
Wild-type	0.13 ± 0.01	1706 ± 534	0.34 ± 0.05	14.7 ± 3.9	7.62 ± 0.78	0.65 ± 0.08	0.46 ± 0.18
S123A (TM3)	0.10 ± 0.03	337 ± 103*	0.39 ± 0.11	24.8 ± 6.4	11.4 ± 1.29	1.45 ± 0.24	1.10 ± 0.26
S127A (TM3)	0.09 ± 0.01	621 ± 153	0.29 ± 0.04	12.4 ± 5.0	2.53 ± 0.48*	0.67 ± 0.09	0.35 ± 0.02
N175A (TM4)	0.17 ± 0.02	1054 ± 204	1.26 ± 0.27**	13.0 ± 4.4	5.82 ± 1.06	0.82 ± 0.09	5.84 ± 0.75**
H208A (TM5)	0.29 ± 0.01**	1867 ± 256	1.25 ± 0.23*	46.5 ± 9.3	1.96 ± 0.31*	0.91 ± 0.10	0.88 ± 0.16
F257A (TM6)	0.09 ± 0.01	2576 ± 343	0.80 ± 0.17	50.2 ± 16.4	14.2 ± 2.81*	0.93 ± 0.24	3.32 ± 0.44**
W264A (TM6)	0.06 ± 0.01*	75 ± 16**	0.38 ± 0.11	356.6 ± 120.3**	3.27 ± 1.16	4.39 ± 0.45**	1.11 ± 0.17
S293A (TM7)	0.10 ± 0.03	1878 ± 312	0.32 ± 0.10	23.2 ± 8.4	3.92 ± 1.42	1.03 ± 0.31	0.40 ± 0.02

HEK-293 cells were transiently transfected with the FLAG-tagged WT or mutant hMT_2 melatonin receptors. Whole cell lysates were used to determine binding constants in saturation (K_d, B_{max}) and competition (K_i) studies using 2-[^{125}I]-iodomelatonin. In saturation studies, non-specific binding was determined with 1 μM melatonin. Values represent the mean ± SEM in competition (N = 3–6) and saturation (N = 3–4) experiments. Ser 8 in TM3 (S123), Ser 12 in TM3 (S127), Asn 16 in TM4 (N175), His 7 in TM5 (H208), Phe 6 in TM6 (F257), Trp 13 in TM6 (W264), and Ser 6 in TM7 (S293). MLT: melatonin; 4P-ADOT: 4-phenyl-2-acetamidotetraline; NAT: *N*-acetyltryptamine; LUZ: luzindole; 5-MTP: 5-methoxytryptophol. (*) $P < 0.05$, (**) $P < 0.01$, when compared with the WT FLAG-tagged hMT_2 melatonin receptor.

binding affinity (K_i) for melatonin by approximately 4-fold (Table 1 and Fig. 2). Both the models proposed by Grol and Jansen [13] and Navajas *et al.* [11] would predict an interaction between the oxygen of the methoxy group in the melatonin molecule and His 7 in TM5 in the hMT_2 melatonin receptor. While the present results suggest that His 7 in TM5 is critical for melatonin binding, there was no change in affinity for 5-methoxytryptophol suggesting that interaction between His 7 in TM5 and the methoxy group may not be critical in the absence of the *N*-acetyl group (Table 1 and Fig. 2). We also found Asn 16 in TM4 to be involved in melatonin recognition of its receptor as previously reported by Sugden *et al.* [12], who suggested an interaction of this amino acid with the *N*-acetyl group of the melatonin molecule. Mutation of Asn 16 in TM4 significantly decreased the affinity (K_i) of both melatonin (∼4-fold) and 5-methoxytryptophol (∼13-fold) in competition for 2-[^{125}I]-iodomelatonin (Table 1 and Fig. 2). These are the only two ligands possessing a 5-methoxy group suggesting that Asn 16 in TM4 may facilitate binding of the methoxy side chain of the melatonin molecule to the hMT_2 melatonin receptor.

Both Sugden *et al.* [12] and Navajas *et al.* [11] suggested aromatic interactions between the indole ring of the melatonin molecule and aromatic residues within the TM regions of the melatonin receptor. Mutation of the two aromatic residues Phe 6 or Trp 13 in TM6 in the hMT_2 melatonin receptor did not significantly alter the affinity of melatonin, however, it did affect the affinity of other ligands. Mutation of Trp 13 in TM6 significantly decreased the affinity of both 4P-ADOT and *N*-acetyltryptamine by approximately 7- and 24-fold, respectively (Table 1 and Fig. 2). 4P-ADOT contains an aromatic ring on carbon 4 of the amidotetraline ring and thus mutation of Trp 13 in TM6 may have prevented π–π interactions. Similarly, mutation of Phe 6 in TM6 significantly decreased the affinity of both luzindole and 5-methoxytryptophol by approximately 2- and 7-fold, respectively (Table 1 and Fig. 2). Luzindole, like 4P-ADOT, has an aromatic group on carbon 2 and thus Phe 6 in TM6 may provide π–π interactions. Thus, while Trp 13 and Phe 6 in TM6 are not critical for melatonin binding, they appear to be important for binding of other ligands with selectivity for the MT_2 melatonin receptor through aromatic interactions. Interestingly, all three models [11–13] proposed the involvement of conserved serines in melatonin recognition of its receptor. Our results suggest that neither Ser 8 or Ser 12 in TM3, or Ser 6 in TM7 in the hMT_2 melatonin receptor are important for melatonin binding (Table 1 and Fig. 2). Taken together, these results suggest that only residues His 7 in TM5 and Asn 16 in TM4 appear to be important for melatonin recognition of the hMT_2 melatonin receptor. It must be acknowledged, however, that the three molecular models by Sugden *et al.* [12], Grol and Jansen [13], and Navajas *et al.* [11] are based on both the structure and pharmacology of the MT_1 and/or Mel_{1c} receptors. In native tissues, the level of MT_2 melatonin receptors is too low to be detected with 2-[^{125}I]-iodomelatonin binding [18]. Therefore, these models may not be appropriate to make predictions regarding the molecular structure of the MT_2 melatonin receptor.

Recent studies have identified residues critical for melatonin binding to the MT_1 melatonin receptor of various species, including Gly 20 in TM6 [19,20] and Ser 8 and Ser 12 in TM3 [15] in the human MT_1 melatonin receptor and Val 4 and His 7 in TM5 [14] in the ovine MT_1 melatonin receptor. Thus, His 7 in TM5 in both the ovine $Mel_{1aβ}$ receptor [14] and the hMT_2 melatonin receptor (present study) are critical for melatonin binding. In contrast, the conserved serines (Ser 8 and Ser 12 in TM3) are critical for melatonin binding to the MT_1 melatonin receptor but not the MT_2. Additionally, mutation of the conserved Ser 12 in TM3 significantly increased the affinity of the melatonin

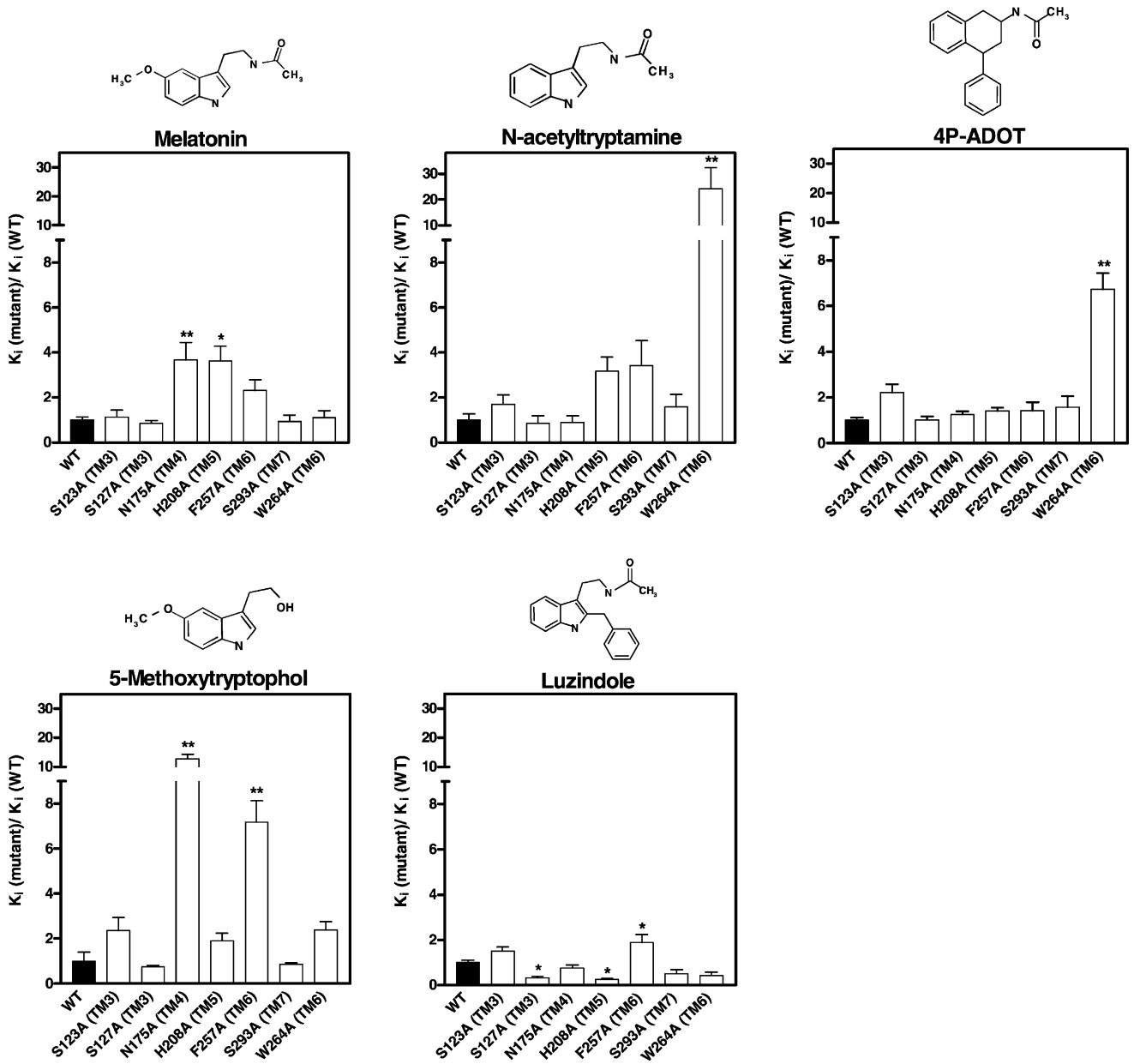

Fig. 2. Fold change in affinity of FLAG-tagged hMT_2 mutant melatonin receptors compared to WT receptors. Affinity constants (K_i) of WT and mutant FLAG-tagged hMT_2 melatonin receptors were determined in competition with several receptor ligands: melatonin, 4P-ADOT, *N*-acetyltryptamine, luzindole, and 5-methoxytryptophol. A ratio of the affinity constants (K_i mutant/K_i WT) was used to determine the fold change in affinity. Ratios are representative of 3–6 independent determinations. Ser 8 in TM3 (S123), Ser 12 in TM3 (S127), Asn 16 in TM4 (N175), His 7 in TM5 (H208), Phe 6 in TM6 (F257), Trp 13 in TM6 (W264), and Ser 6 in TM7 (S293). (*) $P < 0.05$, (**) $P < 0.01$, when compared to the WT FLAG-tagged hMT_2 melatonin receptor.

receptor antagonist luzindole for the MT_2 melatonin receptor (present study) and had no effect in the MT_1 receptor [15]. We conclude that His 7 in TM5 and Asn 16 in TM4 appear critical for melatonin binding to the hMT_2 melatonin receptor, as mutation of these residues decreased the affinity of melatonin to compete with 2-[^{125}I]-iodomelatonin, but also acknowledge that other amino acids not mutated in this study play a role in melatonin binding to the hMT_2 melatonin receptor. Furthermore, Asn 16 in TM4 may facilitate interaction between the methoxy group of the melatonin molecule and the hMT_2 melatonin receptor. Thus, the binding pockets of the MT_1 and MT_2 melatonin receptors appear to share a common histidine residue (His 7 in TM5) but also have distinct residues (Ser 8 and 12 in TM3) necessary for ligand binding. These differences in the molecular structure of the binding sites of the MT_1 and MT_2 melatonin receptors could be used in the design of selective and novel molecules with therapeutic potential.

Acknowledgments

We would like to thank Dr. Martina Bertsch for insightful comments on the manuscript. This research

was supported by USPHS Grant MH-42922 to M.L.D., T32 EY07128-06 and F31 MH67320-01 to M.J.G. and NS11061 and T32 AG00260 to F.M.

References

[1] Brzezinski A. Melatonin in humans. N Engl J Med 1997;336:186–95.
[2] Masana MI, Dubocovich ML. Melatonin receptor signaling: finding the path through the dark. Sci STKE 2001;E39:1–5.
[3] Reppert SM, Weaver DR, Ebisawa T. Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. Neuron 1994;13:1177–85.
[4] Reppert SM, Godson C, Mahle CD, Weaver DR, Slaugenhaupt SA, Gusella JF. Molecular characterization of a second melatonin receptor expressed in human retina and brain: the Mel_{1b} melatonin receptor. Proc Natl Acad Sci USA 1995;92:8734–8.
[5] Ebisawa T, Karne S, Lerner MR, Reppert SM. Expression cloning of a high-affinity melatonin receptor from *Xenopus* dermal melanophores. Proc Natl Acad Sci USA 1994;91:6133–7.
[6] Reppert SM, Weaver DR, Cassone VM, Godson C, Kolakowski Jr LF. Melatonin receptors are for the birds: molecular analysis of two receptor subtypes differentially expressed in chick brain. Neuron 1995;15:1003–15.
[7] Gershengorn MC, Osman R, Ho HH. Minireview: insights into G protein-coupled receptor function using molecular models. Endocrinology 2001;142:2–10.
[8] Ballesteros JA, Shi L, Javitch JA. Structural mimicry in G protein-coupled receptors: implications of the high-resolution structure of rhodopsin for structure–function analysis of rhodopsin-like receptors. Mol Pharmacol 2001;60:1–19.
[9] Pebay-Peyroula E, Rummel G, Rosenbusch JP, Landau EM. X-ray structure of bacteriorhodopsin at 2.5 angstroms from microcrystals grown in lipidic cubic phases. Science 1997;277:1676–81.
[10] Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M. Crystal structure of rhodopsin: a G protein-coupled receptor. Science 2000;289:739–45.
[11] Navajas C, Kokkola T, Poso A, Honka N, Gynther J, Laitinen JT. A rhodopsin-based model for melatonin recognition at its G protein-coupled receptor. Eur J Pharmacol 1996;304:173–83.
[12] Sugden D, Chong NW, Lewis DF. Structural requirements at the melatonin receptor. Br J Pharmacol 1995;114:618–23.
[13] Grol CJ, Jansen JM. The high affinity melatonin binding site probed with conformationally restricted ligands—II. Homology modeling of the receptor. Bioorg Med Chem 1996;4:1333–9.
[14] Conway S, Canning SJ, Barrett P, Guardiola-Lemaitre B, Delagrange P, Morgan PJ. The roles of valine 208 and histidine 211 in ligand binding and receptor function of the ovine $Mel_{1a\beta}$ melatonin receptor. Biochem Biophys Res Commun 1997;239:418–23.
[15] Conway S, Mowat ES, Drew JE, Barrett P, Delagrange P, Morgan PJ. Serine residues 110 and 114 are required for agonist binding but not antagonist binding to the melatonin MT_1 receptor. Biochem Biophys Res Commun 2001;282:1229–36.
[16] Mseeh F, Gerdin MJ, Dubocovich ML. Identification of cysteines involved in ligand binding to the human melatonin MT_2 receptor. Eur J Pharmacol 2002;449:29–38.
[17] Cheng Y, Prusoff WH. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzymatic reaction. Biochem Pharmacol 1973;22:3099–108.
[18] Dubocovich ML, Masana MI, Iacob S, Sauri DM. 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 ML_1 presynaptic heteroreceptor. Naunyn Schmiedebergs Arch Pharmacol 1997;355:365–75.
[19] Gubitz AK, Reppert SM. Chimeric and point-mutated receptors reveal that a single glycine residue in transmembrane domain 6 is critical for high affinity melatonin binding. Endocrinology 2000;141:1236–44.
[20] Conway S, Drew JE, Mowat ES, Barrett P, Delagrange P, Morgan PJ. Chimeric melatonin MT_1 and melatonin-related receptors. Identification of domains and residues participating in ligand binding and receptor activation of the melatonin MT_1 receptor. J Biol Chem 2000;275:20602–9.