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Molecular characterization of a second melatonin receptor expressed in human retina and brain: The Mel_{1b} melatonin receptor

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ABSTRACT **A G protein-coupled receptor for the pineal hormone melatonin was recently cloned from mammals and designated the Mel_{1a} melatonin receptor. We now report the cloning of a second G protein-coupled melatonin receptor from humans and designate it the Mel_{1b} melatonin receptor. The Mel_{1b} receptor cDNA encodes a protein of 362 amino acids that is 60% identical at the amino acid level to the human Mel_{1a} receptor. Transient expression of the Mel_{1b} receptor in COS-1 cells results in high-affinity 2-[^{125}I]iodomelatonin binding (K_d = 160 ± 30 pM). In addition, the rank order of inhibition of specific 2-[^{125}I]iodomelatonin binding by eight ligands is similar to that exhibited by the Mel_{1a} melatonin receptor. Functional studies of NIH 3T3 cells stably expressing the Mel_{1b} melatonin receptor indicate that it is coupled to inhibition of adenylyl cyclase. Comparative reverse transcription PCR shows that the Mel_{1b} melatonin receptor is expressed in retina and, to a lesser extent, brain. PCR analysis of human–rodent somatic cell hybrids maps the Mel_{1b} receptor gene (*MTNR1B*) to human chromosome 11q21–22. The Mel_{1b} melatonin receptor may mediate the reported actions of melatonin in retina and participate in some of the neurobiological effects of melatonin in mammals.**

The pineal hormone melatonin can influence the timing of mammalian circadian rhythms, and it regulates the reproductive alterations that occur in response to changes in day length in seasonally breeding mammals (1–3). Melatonin appears to elicit these neurobiological responses through pharmacologically specific, guanine nucleotide binding protein (G protein)-coupled receptors. Receptors for melatonin were initially identified by ligand-binding studies and *in vitro* autoradiography using the biologically active agonist 2-[^{125}I]iodomelatonin (^{125}I-Mel) (4–6).

Recently, a high-affinity melatonin receptor was cloned by expression cloning from *Xenopus laevis* dermal melanophores (7). Subsequently, a high-affinity melatonin receptor that was 60% identical at the amino acid level with the frog receptor was cloned from several mammals, including humans, by using a PCR approach based on the frog sequence (8). The mammalian receptors show >80% amino acid identity with each other and thus appear to be species homologs of the same receptor (8), designated the Mel_{1a} melatonin receptor (9). The Mel_{1a} receptor is expressed in the hypophyseal pars tuberalis (PT) and hypothalamic suprachiasmatic nuclei (SCN), prominent sites of ^{125}I-Mel binding and presumed sites of the reproductive and circadian actions of melatonin, respectively (ref. 8; for review see ref. 10).

We now report the isolation and characterization of clone H7,¶ which encodes a second G protein-coupled melatonin receptor from humans. This clone is expressed in retina and brain, and it exhibits binding and functional characteristics that are very similar to those of the Mel_{1a} receptor.

MATERIALS AND METHODS

PCR. For PCR with degenerate primers, genomic DNA was subjected to 30 cycles of amplification with 200 nM (final concentration) each of two oligonucleotide primers. Each reaction cycle consisted of incubations at 94°C for 45 sec, 45°C for 2 min, and 72°C for 2 min, with AmpliTaq DNA polymerase (Perkin–Elmer/Cetus). For PCR with specific primers, genomic DNA or first-strand cDNA was subjected to 25 to 35 cycles of amplification using incubations at 94°C for 45 sec, 60°C for 45 sec, and 72°C for 2 or 3 min.

Library Screening. A human genomic library in EMBL-3 SP6/T7 (Clontech) was plated and transferred to Colony Plaque Screen filters (New England Nuclear). The filters were screened under conditions of either high or reduced stringency, as previously described (8). λ phage that hybridized to the probe were plaque-purified.

Expression Studies. COS-1 and NIH 3T3 cells were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, penicillin (50 units/ml), and streptomycin (50 μg/ml), in a 5% CO_2/95% air atmosphere at 37°C.

For ligand-binding studies, melatonin receptor cDNAs in pcDNA3 were introduced into COS-1 cells by using the DEAE-dextran method (11). Three days after transfection, medium was removed, and crude membranes were prepared. Binding assays were performed in duplicate in a final volume of 200 μl, at 37°C for 60 min. Nonspecific binding was defined by 10 μM melatonin. Protein was measured by the method of Bradford (12). Binding data were analyzed by the LIGAND Program of Munson and Rodbard (13).

cAMP Studies. For cAMP studies, the receptor cDNA in pcDNA3 was introduced into NIH 3T3 cells by using Lipofectamine (GIBCO/BRL). Transformed NIH 3T3 cells resistant to Geneticin (G418; 1.0 mg/ml; GIBCO/BRL) were isolated, and single colonies expressing ^{125}I-Mel binding (>200 fmol/mg of total cellular protein) were isolated.

Transformed NIH 3T3 cells were plated in triplicate on 35-mm dishes. Forty-eight hours later, the cells were washed (twice) with DMEM and preincubated with 250 μM 3-isobutyl-1-methylxanthine (IBMX) in DMEM for 10 min at 37°C. Cells were then incubated with or without drugs in DMEM

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Abbreviations: ^{125}I-Mel, 2-[^{125}I]iodomelatonin; PT, hypophyseal pars tuberalis; SCN, hypothalamic suprachiasmatic nuclei; RT, reverse transcription.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. U25341).

with 250 μM IBMX for 10 min at 37°C. At the end of treatment, the cells were processed as previously described (8) and assayed for cAMP by radioimmunoassay (New England Nuclear).

Comparative Reverse Transcription (RT)-PCR Analysis. A comparative RT-PCR assay was performed by using a modification of a previously described procedure (14). Poly(A)$^+$ RNA was purchased from Clontech and 2 μg from each tissue was primed with random hexamers and reverse transcribed as previously described (15). The cDNA was subjected to 25 cycles of amplification with 200 nM each of two specific primers.

The H7 and Mel_{1a} receptor primers were designed so that they would amplify cDNA across the intron splice sites in the first cytoplasmic loop. Since the introns for both H7 and the Mel_{1a} receptor genes are large (>8 kb), amplification of the appropriate-sized cDNA fragments would eliminate the possibility of amplification of genomic DNA. The H7 primers were 5′-TCCTGGTGATCCTCTCCGTGCTCA-3′ and 5′-AGCCAGATGAGGCAGATGTGCAGA-3′, and they amplified a band of 321 bp. The Mel_{1a} receptor primers were 5′-TCCTGGTCATCCTGTCGGTGTATC-3′ and 5′-CTGCTGTACAGTTTGTCGTACTTG-3′, and they amplified a band of 285 bp. Histone-H3.3 served as a control to verify the amount of template for each sample. The histone H3.3 primers were 5′-GCAAGAGTGCGCCCTCTACTG-3′ and 5′-GGCCTCACTTGCCTCCTGCAA-3′, and they amplified a band of 217 bp.

After PCR, the reaction products were subjected to electrophoresis through a 1.5% agarose gel and blotted onto GeneScreen (New England Nuclear). To increase the specificity of the assay, blots were hybridized with 25-mer oligonucleotides, labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase. For each primer pair, the oligonucleotide probes were specific for a sequence of the amplified fragment between the primers. Hybridizing conditions were 45°C overnight in 0.5 M sodium phosphate buffer, pH 7.2/7% SDS/1% bovine serum albumin/1 mM EDTA. The blots were washed twice in 0.2 M sodium phosphate buffer, pH 7.2/1% SDS/1 mM EDTA at 45°C for 30 min.

DNA Sequencing. Nucleotide sequences were analyzed by the dideoxynucleotide chain termination method of Sanger *et al.* (16), using Sequenase (United States Biochemical). Sequencing template was double-stranded plasmid. Primers were synthetic oligonucleotides that were either vector specific or derived from sequence information.

Drugs. ^{125}I-Mel was purchased from New England Nuclear. All drugs used in competition studies were purchased from Sigma or Research Biochemicals (Natick, MA) or were synthesized locally. All other chemicals were purchased from Sigma.

RESULTS

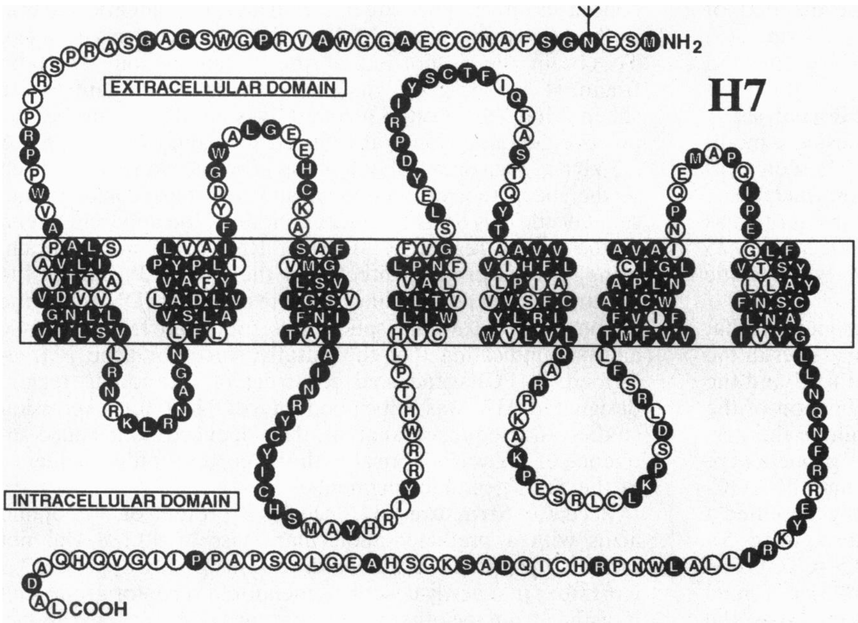
Isolation of a Novel Melatonin Receptor Clone. To clone melatonin receptor subtypes, we used PCR amplification of human genomic DNA with degenerate oligonucleotide primers based on conserved amino acid residues in the third and sixth transmembrane domains of the *Xenopus* melatonin receptor and mammalian Mel_{1a} melatonin receptor. A cDNA fragment (364 bp) was found by sequence analysis to be 60% identical at the amino acid level with either the human Mel_{1a} melatonin receptor or the *Xenopus* melatonin receptor. This PCR fragment was random prime labeled and used to probe a human genomic library at high stringency. A 6-kb *Sac* I fragment of a genomic clone that hybridized to the PCR-generated cDNA fragment was subcloned and partially sequenced. This fragment contained the 3′ end of the putative coding region and extended 5′ to the GN sequence in the first cytoplasmic loop, in which an apparent intron occurred; a consensus intron splice site occurs at a location identical to that in the human and sheep Mel_{1a} melatonin receptor genes (8). To obtain the 5′ portion of the coding region, a 160-bp fragment encoding the first transmembrane domain of the sheep Mel_{1a} melatonin receptor was used to reprobe the positive genomic clones at reduced stringency (see ref. 8). A 2.3-kb *Sac* I fragment of one of the genomic clones hybridized to the sheep receptor fragment and was thus subcloned and sequenced. This *Sac* I fragment contained the apparent 5′ end of the coding region. RT-PCR of RNA from human brain, using specific primers directed at the 5′ and 3′ ends of the putative coding region, amplified the expected cDNA with the intron removed at the splice sites predicted from genomic analysis, indicating that the putative receptor gene is transcribed. A PCR-generated construct of the coding region, designated H7, was subcloned in pcDNA3 for expression studies and sequence analysis. The deduced amino acid sequence of H7 was identical with the corresponding sequence of the *Sac* I genomic fragments.

Receptor Structure. H7 encodes a protein of 362 amino acids with a predicted molecular mass of 40,188 Da, not including posttranslational modifications (Fig. 1 *Upper*). H7 is a member of a newly described melatonin receptor group that is distinct from the other receptor groups (e.g., biogenic amine, neuropeptide, and photopigment receptors) that make up the prototypic G protein-coupled receptor family (7, 8). Unique features of this group include an NRY sequence just downstream from the third transmembrane domain (rather than DRY) and a NAXXY sequence in transmembrane 7 (rather than NPXXY) (Fig. 1 *Lower*). In addition, H7, the mammalian Mel_{1a} melatonin receptors, and the *Xenopus* melatonin receptor all have a CYICHS sequence immediately downstream from NRY in the third cytoplasmic loop which is a consensus site for cytochrome *c* family heme binding (17). The functional significance of this sequence is not yet known.

Pairwise comparisons of H7, the human Mel_{1a} melatonin receptor, and the *Xenopus* melatonin receptor reveal ≈60% amino acid identity for any pair of the three sequences (Fig. 1). Within the transmembrane domains the amino acid identity among any two of the three sequences is 73%. Within the amino-terminal region there is one consensus site for asparagine-linked glycosylation for H7 (Fig. 1 *Lower*). Because of the way H7 was isolated, the possibility of additional upstream translation start sites cannot be excluded.

Binding and Pharmacological Characterization. To determine whether H7 encodes a melatonin receptor, binding and pharmacological properties were examined by transiently expressing H7 in COS-1 cells. For comparison, binding and pharmacology of COS-1 cells transiently expressing the human Mel_{1a} receptor were assessed in parallel. Scatchard transformation of the saturation data showed that COS-1 cells transfected with either receptor cDNA bind ^{125}I-Mel with high affinity. The K_d of cells expressing H7 was 160 ± 30 pM (mean ± SEM; n = 5 experiments) (Fig. 2). This value represents an affinity 1/4 that of the human Mel_{1a} receptor (K_d = 65 ± 6 pM; n = 3) found in parallel experiments. The B_{max} values were 2.7 ± 0.1 pmol/mg of membrane protein for H7 and 2.8 ± 0.4 pmol/mg of membrane protein for the human Mel_{1a} melatonin receptor.

The pharmacological characteristics for inhibition of specific ^{125}I-Mel binding in transfected COS-1 cells were next examined for H7 and compared with those of the human Mel_{1a} receptor (Fig. 3; Table 1). For H7, the rank order of inhibition of specific ^{125}I-Mel binding by six ligands was 2-iodomelatonin > 2-phenylmelatonin > S-20098 > 6-chloromelatonin > melatonin > *N*-acetyl-5-hydroxytryptamine (Fig. 3 *Upper*; Table 1). Micromolar concentrations of prazosin or 5-hydroxytryptamine did not inhibit specific ^{125}I-Mel binding. The rank order of inhibition of specific ^{125}I-Mel binding for H7 was very similar to that found in parallel experiments for the human

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                                                                 I                                  II
H7         MSENGSFANCCEAGGWAVRPGWSGAGSARPSRTPRPPWVAPALSAVLIVTTAVDVVGNLLVILSVLRNRKLRNAGNLFLVSLALADLVVAFYPYPLILVAIFYDGWAL 108
Human 1a          MQGNGSALPNASQPVLRGDGA...RPSWLASALACVLIFTIVVDILGNLLVILSVYRNKKLRNAGNIFVVSLAVADLVVAIYPYPLVLMSIFNNGWNL  95
Xenopus     MMEVNSTCLDCRTPGTIRTEQDAQDSASQG.......LTSALAVVLIFTIVVDVLGNILVILSVLRNKKLQNAGNLFVVSLSIADLVVAVYPYPVILIAIFQNGWTL 100

Consensus          ------------------------------AL--VLI-T--VD--GN-LVILSV-RN-KL-NAGN-F-VSL--ADLVVA-YPYP--L--IF--GW-L

                  III                               IV                                     V
H7         GEEHCKASAFVMGLSVIGSVFNITAIAINRYCYICHSMAYHRIYRRWHTPLHICLIWLLTVVALLPNFFVGSLEYDPRIYSCTFIQTASTQYTAAVVVIHFLLPIAVVSFC 219
Human 1a   GYLHCQVSGFLMGLSVIGSIFNITGIAINRYCYICHSLKYDKLYSSKNSLCYVLLIWLLTLAAVLPNLRAGTLQYDPRIYSCTFAQSVSSAYTIAVVVFHFLVPMIIVIFC 206
Xenopus    GNIHCQISGFLMGLSVIGSVFNITAIAINRYCYICHSLRYDKLYNQRSTWCYLGLTWILTIIAIVPNFFVGSLQYDPRIFSCTFAQTVSSSYTITVVVVHFIVPLSVVTFC 211

Consensus  G--HC--S-F-MGLSVIGS-FNIT-IAINRYCYICHS--Y---Y---------L-W-LT--A--PN---G-L-YDPRI-SCTF-Q--S--YT--VVV-HF--P---V-FC

                                                 VI                            VII
H7         YLRIWVLVLQARRKAKPESRLCLKPSDLRSFLTMFVVFVIFAICWAPLNCIGLAVAINPQEMAPQIPEGLFVTSYLLAYFNSCLNAIVYGLLNQNFRREYKRILLALWNPR 330
Human 1a   YLRIWILVLQVRQRVKPDRKPKLKPQDFRNFVTMFVVFVLFAICWAPLNFIGLAVASDPASMVPRIPEWLFVASYYMAYFNSCLNAIIYGLLNQNFRKEYRRIIVSLCTAR 317
Xenopus    YLRIWVLVIQVKHRVRQDFKQKLTQTDLRNFLTMFVVFVLFAVCWAPLNFIGLAVAINPFHVAPKIPEWLFVLSYFMAYFNSCLNAVIYGVLNQNFRKEYKRILMSLLTPR 322

Consensus  YLRIW-LV-Q-----------L---D-R-F-TMFVVFVLFA-CWAPLN-IGLAVA--P----P-IPE-LFV-SY--AYFNSCLNA--YG-LNQNFR-EY-RI---L---R


H7         HCIQDASKGSHAEGLQSPAPPIIGVQHQADAL 362
Human 1a   VFFVDSSNDVADRVKWKPSPLMTNNNVVKVDSV 350
Xenopus    LLFLDTSRGGTEGLKSKPSPAVTNNNQADMLGEARSLWLSRRNGAKMVIIIRPRKAQIAIIHQIFWPQSSWATCRQDTKITGEEDGCRELCKDGISQR 420

Consensus  ----D-S----------P-P------------
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FIG. 1. Structure of the H7 protein. (*Upper*) Predicted membrane topology of the H7 protein. Y, Potential asparagine-linked glycosylation site. Amino acids that are shaded are identical between H7 and the human Mel_{1a} melatonin receptor. (*Lower*) Comparison of the deduced amino acid sequence of H7 (GenBank accession no. U25341) with the human Mel_{1a} melatonin receptor (U14108) and the *Xenopus* melatonin receptor (U09561). To maximize similarities, gaps (dots) have been introduced into the three sequences. The seven presumed transmembrane domains (I–VII) are overlined. Consensus sites for asparagine-linked glycosylation are underlined.

Mel_{1a} melatonin receptor, except that 6-chloromelatonin was 10-fold more potent in inhibiting specific ^{125}I-Mel binding in cells expressing H7 (Fig. 3 *Lower*; Table 1). Thus, H7 encodes a protein with ^{125}I-Mel binding characteristics that are quite similar to those of the Mel_{1a} melatonin receptor.

H7 Inhibits cAMP Accumulation. We examined whether the recombinant receptor encoded by H7 is coupled to inhibition of adenylyl cyclase as is the Mel_{1a} melatonin receptor (8). For these studies, we used clonal lines of NIH 3T3 cells stably transfected with the receptor cDNA in pcDNA3. Melatonin (1 μM) did not increase basal cAMP levels in stably transfected NIH 3T3 cells (data not shown). Melatonin did cause a dose-dependent inhibition of the increase in cAMP accumulation induced by 10 μM forskolin (Fig. 4); the maximal inhibition of forskolin-stimulated cAMP accumulation was at 10^{-8} M melatonin. Melatonin (1 μM) did not inhibit forskolin-stimulated cAMP accumulation in nontransfected cells or cells transfected with vector lacking receptor cDNA (8). Thus, the recombinant melatonin receptor is negatively coupled to the cAMP regulatory system.

Distribution of H7 mRNA. To assess the tissue distribution of H7 mRNA, a 364-bp fragment of the rat homolog of H7 was cloned from rat brain RNA by RT-PCR; the rat cDNA fragment was 81% identical at the amino acid level with H7 (data not shown; GenBank accession no. U28218). *In situ* hybridization using an antisense cRNA probe to the rat fragment did not reveal a hybridization signal in PT or SCN, sites which gave a positive hybridization signal in the same *in situ* run when an antisense cRNA probe to the Mel_{1a} melatonin receptor was used (8).

Because of the apparent low level of receptor transcripts, a comparative RT-PCR assay was used to examine the expression of H7 and the human Mel_{1a} receptor genes in six human tissues (Fig. 5). H7 was expressed in retina, with much lower expression in whole brain and hippocampus. The Mel_{1a} receptor was clearly expressed in whole brain, with just detectable expression in retina and hippocampus. Neither H7 nor Mel_{1a} receptor mRNA was detected in pituitary, liver, or spleen.

Gene Structure and Chromosomal Localization. Restriction endonuclease mapping and PCR analysis of genomic clones

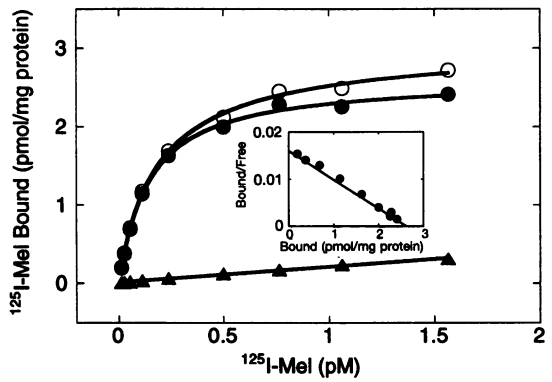

FIG. 2. Expression of H7 in COS-1 cells assayed by ^{125}I-Mel binding. ○, Total binding; ●, specific binding; ▲, nonspecific binding (determined in the presence of 10 μM melatonin). (*Inset*) Scatchard plot of saturation data. The K_d value depicted is 150 pM. The B_{max} value is 2.62 pmol/mg of membrane protein. Data shown are representative of five experiments.

showed that the portion of the gene that encodes H7 is composed of 2 exons, separated by an intron that is ≈9.0 kb in length. Southern analysis of human genomic DNA digested with several different restriction endonucleases was performed by using a PCR fragment of the second exon of H7 as a hybridization probe. Under high-stringency conditions, we observed single-band patterns, suggesting that H7 is a single-copy gene (data not shown).

To localize the gene for H7, an intronic PCR assay was developed that would amplify only the human H7 gene. A panel of 43 human–rodent somatic cell hybrids that contained

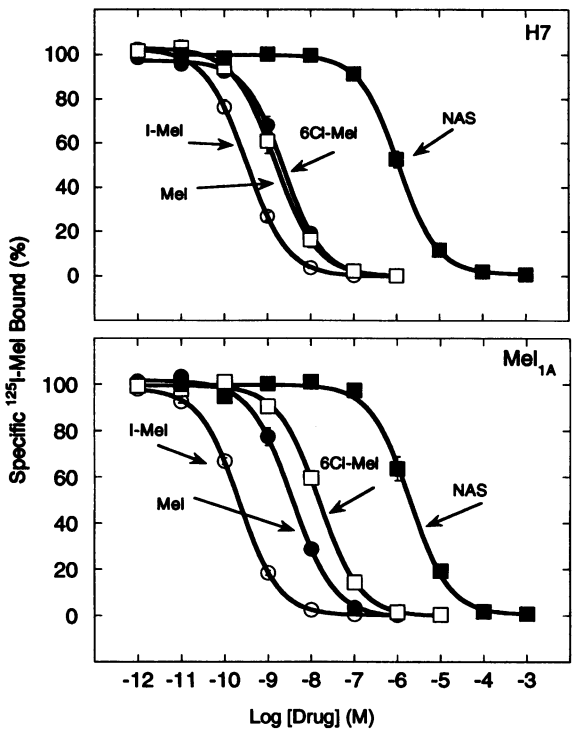

FIG. 3. Competition by various ligands for ^{125}I-Mel binding in COS-1 cells transfected with either H7 or the human Mel$_{1a}$ melatonin receptor cDNA. Cells were incubated with 200 pM (H7) or 100 pM ^{125}I-Mel (Mel$_{1a}$ receptor) and various concentrations of 2-iodomelatonin (I-Mel), melatonin (Mel), 6-chloromelatonin (6Cl-Mel), or *N*-acetyl-5-hydroxytryptamine (NAS). Nonspecific binding was determined in the presence of 10 μM melatonin. The data shown are mean values of three to five experiments for each drug. K_i values are listed in Table 1.

Table 1. Competition of various ligands for specific ^{125}I-Mel binding in COS-1 cells transfected with either H7 or the Mel$_{1a}$ receptor cDNA

Compound	K_i, nM		Ratio
	H7	Mel$_{1a}$	(Mel$_{1a}$/H7)
2-Iodomelatonin	0.17 ± 0.02	0.09 ± 0.01	0.5
2-Phenylmelatonin	0.26 ± 0.06	0.21 ± 0.06	0.8
S20098	0.23 ± 0.04	0.72 ± 0.11	3.1
6-Chloromelatonin	0.66 ± 0.04	6.78 ± 0.91	10.3
Melatonin	1.11 ± 0.13	1.48 ± 0.21	1.3
NAS	595 ± 127	986 ± 137	1.6
5-HT	>10,000	>10,000	—
Prazosin	>10,000	>10,000	—

K_i values are mean ± SEM of three to five experiments for each drug. NAS, *N*-acetyl-5-hydroxytryptamine; 5-HT, 5-hydroxytryptamine.

defined overlapping subsets of human chromosomes was screened (refs. 18 and 19; NIGMS Mapping Panel no. 2, Coriell Institute, Camden, NJ). When primer 5′-CTGTGCCTCTAAGAGCCACTTGGTTTC-3′ and primer 5′-TATTGAAGACAGAGCCGATGACGCTCA-3′ were used, PCR amplified a single band only in those cell lines containing human chromosome 11. The H7 receptor gene was further localized to band 11q21–22 by PCR screening of a panel of somatic cell hybrids containing various deletion fragments of human chromosome 11 (20). The appropriate band was amplified in hybrid lines J1-7, J1-8, J1-10, J1-11, J1-23, P3-27A, and R28-4D, but not in lines J1-1 and J1-44. The gene encoding H7 has been given the designation *MTNR1B*.

DISCUSSION

H7 encodes a newly discovered member of the G protein-coupled melatonin receptor group. Transfection of COS-1 cells with the receptor cDNA results in transient expression of receptors that bind ^{125}I-Mel with high affinity. Specific ^{125}I-Mel binding in transfected COS-1 cells is inhibited by eight ligands in a rank order and potency that are very similar to those found for the recombinant human Mel$_{1a}$ receptor. Melatonin inhibits forskolin-stimulated cAMP accumulation in NIH 3T3 cells stably transfected with H7. Finally, mRNA encoding H7 is expressed in retina and to a lesser extent brain. The similarities of the binding and functional characteristics of H7 to the Mel$_{1a}$ melatonin receptor lead us to designate H7 the Mel$_{1b}$ melatonin receptor.

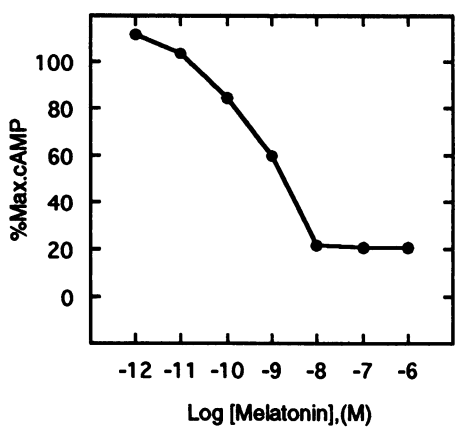

FIG. 4. Melatonin inhibition of forskolin-stimulated cAMP accumulation in NIH 3T3 cells stably transfected with H7. The 100% value is the mean cAMP value induced with 10 μM forskolin. The data shown are mean values of two experiments.

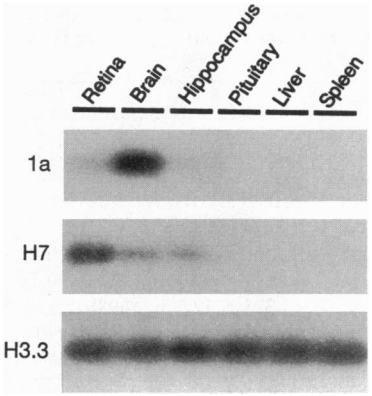

FIG. 5. Comparative RT-PCR analysis of H7 and Mel_{1a} receptor gene expression in six human tissues. Brain, whole brain; H3.3, histone H3.3. An identical pattern of expression was observed in a replication of the experiment shown.

One feature that distinguishes the Mel_{1b} receptor from the Mel_{1a} receptor is its tissue distribution. The expression of the Mel_{1b} receptor in retina suggests that melatonin may exert its effects on mammalian retinal physiology through this receptor. Melatonin inhibits the Ca^{2+}-dependent release of dopamine in rabbit retina through activation of receptors with pharmacologic specificity comparable with that reported here for the Mel_{1b} receptor (4, 21). Melatonin appears to act in the retina to affect several light-dependent functions, including photopigment disc shedding and phagocytosis (22, 23). It will be important to determine the precise anatomical distribution of the Mel_{1b} receptor within mammalian retina and to develop specific pharmacological tools to probe the functions of this receptor in retina.

Our previous studies have shown that the Mel_{1a} melatonin receptor is expressed in SCN and PT (8), sites felt to be involved in the circadian and reproductive functions of melatonin, respectively (10). The discovery of a Mel_{1b} receptor which has binding and functional characteristics similar to those of the Mel_{1a} receptor makes it conceivable that the Mel_{1b} receptor also participates in the circadian and/or reproductive actions of melatonin. Even though Mel_{1b} receptor mRNA is not detectable by *in situ* hybridization in rat SCN or PT, it may be present and functional in these or other neural sites at levels not detectable by using standard detection methods.

A second distinguishing feature of the Mel_{1b} receptor is its chromosome location. The Mel_{1b} melatonin receptor maps to human chromosome 11q21–22, a region syntenic to mouse chromosome 9 in the region of the D_2-dopamine receptor (*Drd2*) and thymus cell antigen 1 (*Thy1*) loci (24, 25). This contrasts with the Mel_{1a} melatonin receptor, which maps to human chromosome 4q35.1 and mouse chromosome 8 (9). Thus, these two structurally and functionally related melatonin receptors did not evolve by simple tandem duplication of an ancestral gene, but other mechanisms, such as duplication and chromosomal rearrangement, were involved. The human and mouse Mel_{1b} receptor genes are located in the same regions as those reported for two other G protein-coupled receptors, the D_2-dopamine receptor, as already mentioned, and an orphan receptor that is most homologous to the interleukin 8, type 1, receptor (26). To our knowledge, no retinal disorders have been mapped to these areas in humans or mice.

An interesting feature common to both the Mel_{1a} and Mel_{1b} receptor genes is the conserved position of the intron splice site in the first cytoplasmic loop. An intron at such a location could lead to alternative splice forms of either of these receptors, thereby altering receptor structure and potentially function. Splice variants have been reported for the D_2-dopamine receptor in the third intracellular loop that are involved in alterations in the specificity of G protein coupling and for other G protein-coupled receptors (27, 28). However, extensive RT-PCR analysis of RNA from several mammalian tissues has not yet given any indication that splice variants exist for either the Mel_{1a} or Mel_{1b} receptors.

The discovery of a second member of the G protein-coupled melatonin receptor family shows that at least two distinct genes have evolved to subserve melatonin's functions. Devising selective molecular lesions and developing selective pharmacological agents should help elucidate the contributions of each of these two receptors to the physiology of melatonin in mammals.

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