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Molecular cloning and functional expression of 5-HT_{1E}-like rat and human 5-hydroxytryptamine receptor genes

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ABSTRACT **Sequential polymerase chain reaction experiments were performed to amplify a unique sequence representing a guanine nucleotide-binding protein (G-protein)-coupled receptor from rat hypothalamic cDNA. Degenerate oligonucleotides corresponding to conserved amino acids from transmembrane domains III, V, and VI of known receptors [5-HT_{1A}, 5-HT_{1C}, and 5-HT_2; 5-HT is serotonin (5-hydroxytryptamine)] were used as primers for the sequential reactions. The resulting product was subcloned and used to screen a rat genomic library to identify a full-length clone (MR77) containing an intronless open reading frame encoding a 366-amino acid seven-transmembrane domain protein. The human homolog was isolated, and its encoded protein had 93% overall amino acid identity with the rat sequence. Within the conserved transmembrane domains, the sequences exhibit approximately 52%, 59%, 65%, and 68% amino acid identity with the known rat 5-HT_{1A}, rat 5-HT_{1B}, rat 5-HT_{1D}, and human 5-HT_{1E} receptors, respectively. MR77 was subcloned into a eukaryotic expression vector system and expressed in CosM6 cells. Studies on broken cell preparations indicate that the expressed receptor exhibits ^{125}I-labeled *d*-lysergic acid diethylamide (LSD) binding that can be displaced by serotonin but not by other biogenic amines. The specific binding is displaced by the selective 5-HT_{1D} agonist sumatriptan but not by the mixed 5-$HT_{1A/1D}$ agonist 5-carboxyamidotryptamine. ^{125}I-labeled LSD binding was competitively antagonized by the ergot alkaloids methysergide and ergotamine. HeLa cells transfected with the MR77 gene exhibited inhibition of adenylate cyclase in response to serotonin. MR77 is expressed at low levels throughout the brain, with the greatest expression in the cortex, hippocampus, and striatum. MR77 thus represents a 5-HT receptor of the 5-HT_1 class, and we propose that, based on the pharmacological characterization, MR77 represents an additional 5-HT_{1E}-like receptor.**

Serotonin (5-hydroxytryptamine, 5-HT) is a biogenic amine neurotransmitter found in both the central and peripheral nervous systems (for review, see ref. 1). A majority of serotonin-containing neurons in the central nervous system have cell bodies located in association with the raphe nuclei in the midline area of the brainstem. These cells send projections to the cortex, several limbic structures (hypothalamus, hippocampus, basal ganglia, and amygdala) as well as the lateral geniculate and superior colliculus. Serotonergic transmission through these pathways is thought to be involved with a variety of behaviors and disorders including anxiety, sleep regulation, aggression, and depression. To understand the effects of current drug therapies and also develop additional selective drugs, the receptor systems involved in serotonergic transmission along these pathways must be elucidated.

Pharmacological experiments have suggested that the actions of serotonin are mediated by multiple types of serotonin receptors, each having a distinct profile of activity with respect to serotonin-related drugs (2). Several serotonin receptors have been identified by cDNA cloning and have been shown to be guanine nucleotide-binding protein (G-protein)-coupled molecules with the putative seven-transmembrane domain structure characteristic of many receptors, including the prototypes rhodopsin and the β-adrenergic receptors (3). Members of the serotonin receptor family have been subdivided on the basis of either affinities for serotonin or sequence similarities. Members of the 5-HT_1 subgroup are characterized by their high affinities for serotonin, whereas 5-HT_2 receptors exhibit low affinity. Currently, five 5-HT_1 and one 5-HT_2 receptor have been identified pharmacologically (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_2). Genomic and/or cDNA clones for all of these receptors have been isolated, and their sequences have been determined (4–11).

In an attempt to identify other receptors that belong to the family of 5-HT receptors, we used a strategy based upon similarities shared among known members of the family. We designed highly degenerate oligonucleotides corresponding to conserved amino acid sequences in putative transmembrane domains III, V, and VI of the 5-HT_{1A}, 5-HT_{1C}, and 5-HT_2 receptors. These oligonucleotides were used in sequential polymerase chain reactions (PCR) with rat hypothalamus cDNA as the template to generate a series of clones. Here we report that one of these clones and its human homolog¶ correspond to a 5-HT receptor that is more closely related in sequence to the recently cloned human S31 receptor (5-HT_{1E}) than any previously described 5-HT receptor but clearly is a new member of the 5-HT_1 receptor family. Initial pharmacological characterization of this receptor, which is negatively coupled to adenylate cyclase, is similar to but distinct from that described previously for the 5-HT_{1E} receptor (9, 12, 13), and therefore we propose that MR77 belongs to a new subgroup of 5-HT_{1E}-like receptors.

MATERIALS AND METHODS

PCR Cloning, Library Screening, and Sequence Analysis. Poly(A)-enriched RNA was isolated from dissected rat hypothalami using Micro-FastTrack kits (Invitrogen, San Diego) and used as template for first-strand synthesis of cDNA by Moloney murine leukemia virus reverse transcriptase

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Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); 5-CT, 5-carboxyamidotryptamine; LSD, *d*-lysergic acid diethylamide.

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¶The sequences reported in this paper have been deposited in the GenBank data base (accession nos. L05596 for the rat clone and L05597 for the human clone).

(Stratagene). Oligonucleotides of degenerate sequence were synthesized corresponding to conserved amino acid sequences of portions of putative transmembrane domains III, V, and VI of the 5-HT_{1A} and 5-HT_{1C} receptors [Leu-Cys-Ala-Ile-(Ala or Ser)-Leu-Asp-Arg-Tyr, Phe-(Val or Gly)-Ala-Phe-(Phe or Tyr)-Ile-Pro-Leu, and (Cys or Met)-Trp-(Leu or Cys)-Pro-Phe-Phe-Ile, respectively]. The first PCR reaction (30 cycles of 94°C for 15 sec, 55°C for 30 sec, and 72°C for 30 sec with a Perkin Elmer Cetus 9600 thermal cycler) used oligonucleotides corresponding to transmembrane regions III and VI (10 μg each) with the hypothalamus cDNA as a template. Products >500 bases were isolated by agarose gel electrophoresis and used as templates for a second PCR reaction with oligonucleotides III and V. The resulting products were ligated into pBluescript (Stratagene) and used to transform DH5α bacteria (BRL). Cloned DNA was used to probe both rat and human genomic libraries (Stratagene). All sequencing was performed by the dideoxy-termination method (14) with Sequenase (United States Biochemical). Sequence data were compiled and analyzed by using Genetics Computer Group sequence software (Madison, WI) (15). Nucleotide sequences of primers (5′ to 3′ with N = A, T, G, or C; Y = T or C; H = T, C, or A; W = A or T; S = C or G; R = A or G; M = A or C; D = A, G, or T; and parentheses = different nucleotides in two degenerate sets of oligonucleotides used for primers III and VI) were as follows: III, T-N-T-G-Y-G-C-N-A-T-H-G(W)-C(S)-N-Y-T-N-G-A-Y-M; V, N-A-R-N-G-G-D-A-T-R-W-A-R-A-A-N-G-C-N-M-C-R-A-A; and VI, D-A-T-R-A-A-R-A-A-N-G-G-N(R)-A(C)-R(A)-C-C-A-R(C)-C(A)-A(T).

Expression in COS-M6 Cells. All expression and pharmacological experiments were carried out with the rat MR77 clone. CosM6 cells (a subclonal line of COS-7 cells, obtained from Edith Womack in the laboratory of J. L. Goldstein, University of Texas Health Sciences, Dallas, and generously provided by L. E. Limbird, Vanderbilt University) or HeLa cells were transfected by using the DEAE dextran or calcium phosphate methods, respectively, with plasmids (10 μg of cesium chloride-purified plasmid per 10-cm dish) pDP5HT1a (16), pDP5MR77, or pBC12BIBeta2 (17) (hamster β_2-adrenergic receptor gene). The hamster β_2-adrenergic receptor gene, obtained from ATCC (deposited by R. J. Lefkowitz, Duke University), was subcloned into the pBC12BI eukaryotic expression vector [provided by B. Cullen, Duke University (18)]. Cotransfection experiments used 2 μg of pBC12BIBeta2 combined with 10 μg of either pDP5HT1a (5-HT_{1A}) or pDP5M77 (MR77).

^{125}I-Labeled *d*-Lysergic Acid Diethylamide (LSD) Binding. Approximately 60 hr after transfection, CosM6 cells were removed from dishes by using EDTA (GIBCO) and a cell scraper and then were collected by centrifugation (250 × *g*, 5 min). Pellets were resuspended in 50 mM Tris·HCl (pH 7.6) with a polytron homogenizer, centrifuged for 20 min at 40,000 × *g*, and resuspended in the same buffer (500 μl per 10-cm dish). ^{125}I-LSD (NEN, 2200 Ci/mmol; 1 Ci = 37 GBq) binding was assessed in a 100-μl final volume and contained 0.02% ascorbate, CosM6 membranes (15 μg of protein), 1 nM ^{125}I-LSD (final concentration), and various unlabeled test compounds. Nonspecific binding was determined by using 100 μM serotonin, and typically was <10% of total dpm. After 60 min at 37°C, 2 ml of ice-cold 50 mM Tris·HCl was added to each tube, and bound ligand was isolated on 0.1% polyethyleneimine-soaked GF/B glass fiber filters, washed twice, and assayed for radioactivity. Protein concentration was measured by the Bradford dye binding method (Bio-Rad) (19). Binding data were analyzed by nonlinear regression analysis as described (20).

Cyclic AMP Accumulation. cAMP accumulation was measured by a method similar to that described by Baron and Siegel (21). HeLa cells were transfected by the calcium phosphate method and utilized 64 hr later. The cells were washed twice with 10 ml of Krebs–Ringer buffer (KRB) containing 134 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 2.5 mM $MgSO_4$, 1.18 mM KH_2PO_4, 11.1 mM glucose, 20 mM Hepes·NaOH, and 0.001% phenol red (pH 7.35). KRB containing 4 μCi of [^{3}H]adenine (10 ml per dish) was added, and the dishes were placed in a 37°C air incubator for 2 hr. The dishes were washed once with KRB and once with 10 ml of EDTA, and then the cells were incubated with 10 ml of fresh EDTA for 30 min at 37°C. Cells were resuspended, pooled, and pelleted at 250 × *g* for 5 min. The final pellet was suspended in KRB containing 0.5 mM isobutylmethylxanthine (IBMX) and 0.02% ascorbate, and 400 μl of the cell suspension was dispensed to each assay tube. After a 5-min preincubation at 37°C, the tubes were cooled on ice, various test compounds or KRB/IBMX was added, and the tubes were returned to the 37°C bath for 10 min. The 37°C incubations were ended by placing the tubes on ice and adding 550 μl of ice-cold 10% perchloric acid containing 5500 dpm of [^{14}C]cAMP, 110 μg of unlabeled ATP, 55 μg of unlabeled cAMP, and 0.002% phenol red. Samples were centrifuged at 3700 rpm for 15 min, and the transferred supernatants were neutralized with 50 μl of 10 M KOH. Insoluble $KClO_4$ was removed by centrifugation, and 50-μl aliquots were removed for determination of total radioactivity in each sample. The remaining 1.0 ml was used for determination of cAMP by the sequential Dowex/alumina ion-exchange method (22). Conversion of cell-associated [^{3}H]ATP to [^{3}H]cAMP was expressed as a percentage of total cell-associated radioactivity (from the 50-μl sample) and was normalized to the percentage recovery of [^{14}C]cAMP tracer (generally 25–40%).

Semiquantitative PCR. To determine the tissue distribution of MR77 mRNA expression in rat, we designed a PCR protocol that takes advantage of the intron present in the genomic MR77 clone (Fig. 3*A*). Two oligonucleotide primers were designed—one was the inverse complement of nucleotides 590–615, and the other corresponded to the region of the cDNA 20 nucleotides upstream from the putative splice site. In a PCR assay, these primers amplify a 563-bp fragment from cDNA template but not from genomic DNA. Poly(A)$^+$ RNA was isolated from a variety of dissected rat tissues including several brain regions. First-strand cDNA was synthesized from 1 μg of poly(A)$^+$ RNA from each tissue by using Moloney murine leukemia virus reverse transcriptase. Two microliters of a 1:10 dilution of each cDNA was used as template in a PCR reaction with the primers described above for 30 amplification cycles; 30 cycles was determined to be within the linear amplification range under the conditions used. Equal aliquots (10 μl) of each PCR reaction were subjected to agarose gel electrophoresis. The resulting gel was denatured, neutralized, transferred to nitrocellulose membrane, and probed with a radiolabeled oligonucleotide complementary to nucleotides 364–385 of MR77.

RESULTS AND DISCUSSION

Identification of cDNA and Genomic MR77 Clones. We used sequential amplification of rat hypothalamus cDNAs with nested primers and PCR to identify novel 5-HT receptor-like sequences. The first amplification was performed with degenerate primers (with cloning sites) corresponding to portions of the coding regions of putative transmembrane domains III and VI that are conserved among known catecholamine and serotonin receptors from the G-protein-associated receptor superfamily. Products longer than 500 base pairs (bp) were isolated and used as templates in a second amplification designed to amplify cDNAs encoding proteins closely related to known 5-HT receptors (by using the same transmembrane III degenerate primer and a degenerate primer corresponding to residues in transmembrane V that

are conserved among known 5-HT receptors but not catecholamine receptors). The PCR products were ligated to vector, and bacterial transformation resulted in approximately 1000 colonies, of which >95% hybridized to a mixture of radiolabeled oligonucleotides corresponding to nonconserved regions located between transmembrane domains III and V of the rat 5-HT_{1A} (nucleotides 1002–976), 5-HT_{1C} (nucleotides 879–856), and 5-HT_2 (nucleotides 1515–1488) receptors. Thirty six colonies that showed either minimal or no hybridization were picked and reprobed with the same oligonucleotide mixture, and partial sequences of nonhybridizing clones were determined. From this screening, two distinct cDNA clones were isolated whose sequences had significant similarity to G-protein-associated receptors. One of these, designated MR77, is described in this paper.

We tried unsuccessfully to screen a rat hypothalamus cDNA library with the MR77 PCR product as a probe. Since many neurotransmitter receptor genes of this superfamily do not contain introns, we hypothesized that a genomic clone may contain an intact open reading frame. A rat genomic library was probed with radiolabeled MR77 DNA. This resulted in the isolation and subsequent subcloning of a 2.5-kilobase (kb) fragment that contained an apparently intronless open reading frame of 1098 nucleotides. However, the reading frame upstream from an apparent initiation codon was also open. Therefore, we used an anchor-PCR protocol with an oligonucleotide primer complementary to nucleotides 311–289 and a poly(dC) oligonucleotide to amplify template rat brain cDNA tailed with dG and cloned the largest product. The sequence of this partial MR77 cDNA clone was identical to the genomic clone from nucleotide 97 to nucleotide 311 but was completely different at its 5′ end beginning 41 nucleotides upstream from the presumed initiation codon. The cDNA sequence contained stop codons in frame with the putative coding sequence, suggesting that the assignment of the initiation codon was correct. Upon closer examination of the genomic clone, it was evident that an intron acceptor site was at nucleotide 97 (asterisk at top of Fig. 1). The intron lies upstream from the complete open reading frame encoding the putative receptor. Because the open reading frame encoded a 366-amino acid sequence that is highly related to known receptors throughout its entire length, we concluded that the mRNA contained no further introns.

Predicted Amino Acid Sequence of Rat and Human MR77. The predicted protein contains seven hydrophobic regions as determined by hydropathy calculations (23) (data not shown). In addition, the N-terminal putative extracellular domain has two potential sites for asparagine-linked glycosylation at amino acids 5 and 10 (Fig. 1, black triangles). There are several potential protein kinase C (circles) and calmodulin kinase II phosphorylation (squares) sites in the large intracellular loop between putative transmembrane domains V and VI (Fig. 1). These potential posttranslational modifications are similar to those predicted for a variety of G-protein-coupled receptors (24, 25). MR77 exhibits considerable amino acid homology, particularly within the transmembrane domains, with known 5-HT receptors that are coupled to the inhibition of adenylate cyclase: human S31 (5-HT_{1E}), dog 5-HT_{1D}, rat 5-HT_{1B}, rat 5-HT_{1A}, and rat 5-HT_{1C} with 55%, 48%, 46%, 35%, and 30% overall identity. The primary amino acid sequence of MR77 is most closely related to the recently described human S31 receptor (26), which has been identified as the 5-HT_{1E} receptor (9). We screened a human genomic library with rat MR77 and determined the sequence of the human MR77 homolog, which is 86% and 93% identical to rat MR77 at the nucleic acid and amino acid sequence levels, respectively. The human amino acids that differ from those of rat are shown in italics in Fig. 1. The relationships among the known G-protein-coupled serotonin receptors are graphically illustrated as a dendrogram (Fig. 2). The 5-HT_{1B} and

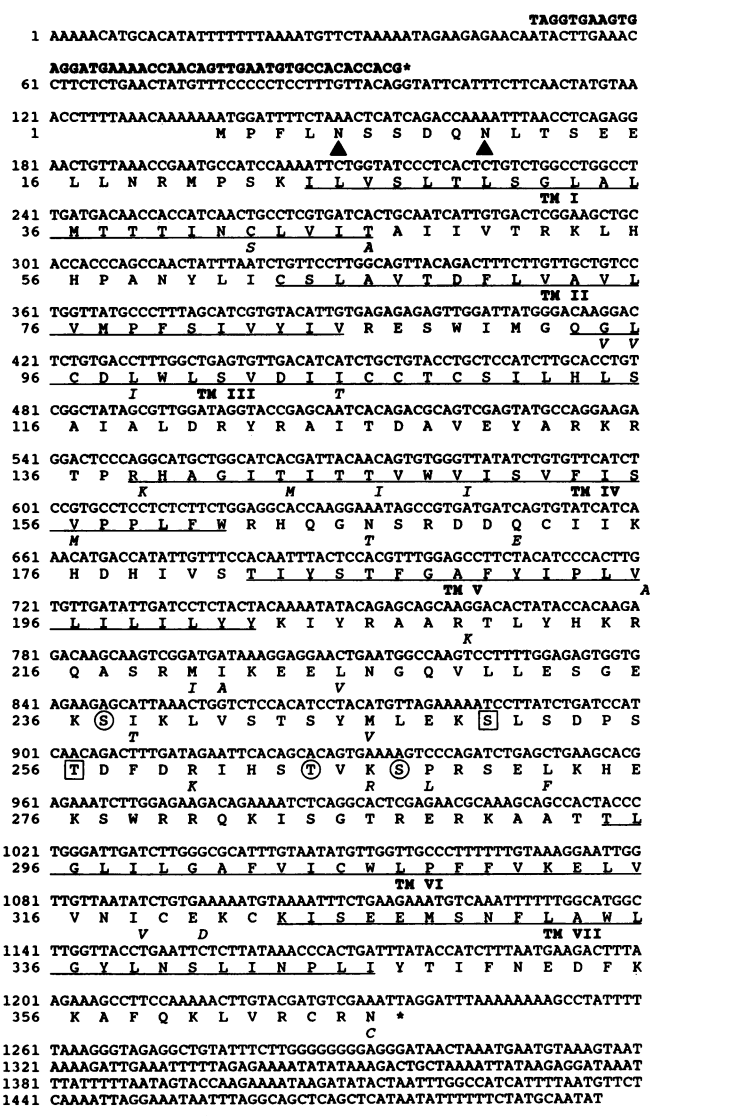

FIG. 1. Nucleotide sequence and predicted amino acid sequence of the rat MR77 genomic clone. The putative transmembrane domains are underlined. Triangles indicate potential N-linked glycosylation sites. The asterisk in line 2 is located above the intron splice junction. The sequence upstream of this site in boldface print represents the MR77 cDNA sequence, which is different from the genomic clone. The cDNA sequence downstream from the asterisk is identical to the genomic clone (not shown). The asterisk at the end of the amino acid sequence signifies the termination codon. Circles and squares indicate amino acids that are consensus phosphorylation sites for protein kinase C and calmodulin kinase II, respectively. Amino acids in italics beneath the rat amino acid sequence represent differences with the human clone.

5-HT_{1D} receptors and the MR77 and 5-HT_{1E} receptors form distinct subfamilies that are more similar to one another than to other receptor types, as determined by amino acid sequence comparisons. In addition, the 5-HT_{1B}, 5-HT_{1D}, MR77, and 5-HT_{1E} receptors are more similar to each other collectively than to 5-HT_{1A}. This may be due in part to the longer putative third intracellular loop in 5-HT_{1A} compared with the other receptors.

Brain Regional Distribution of MR77 mRNA Expression. The tissue distribution of MR77 expression could not be determined by Northern blotting because of the low expression of the mRNA. However, we were able to take advantage of PCR and the fact that the MR77 gene contained an intron in its 5′ untranslated region. Primers were designed so as to amplify MR77, producing a 563-bp fragment from cDNA

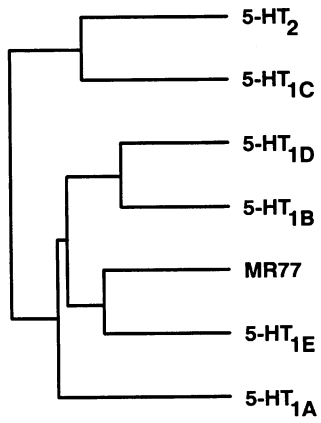

FIG. 2. Dendrogram of the G-protein-linked 5-HT receptor family based on sequence similarity. The relative lengths of the bars are inversely proportional to the similarity between the sequence pairs.

templates but not from genomic DNA (Fig. 3*A*). Therefore, detection of amplified product should be diagnostic for the presence of MR77 mRNA. Equal amounts of cDNA made from mRNA extracted from a variety of rat tissues were subjected to PCR, and a major band migrating at approximately 570 bp was observed with some templates (Fig. 3*B*). The 570-bp PCR product was subcloned, and its sequence was shown to be identical to the appropriate region of the

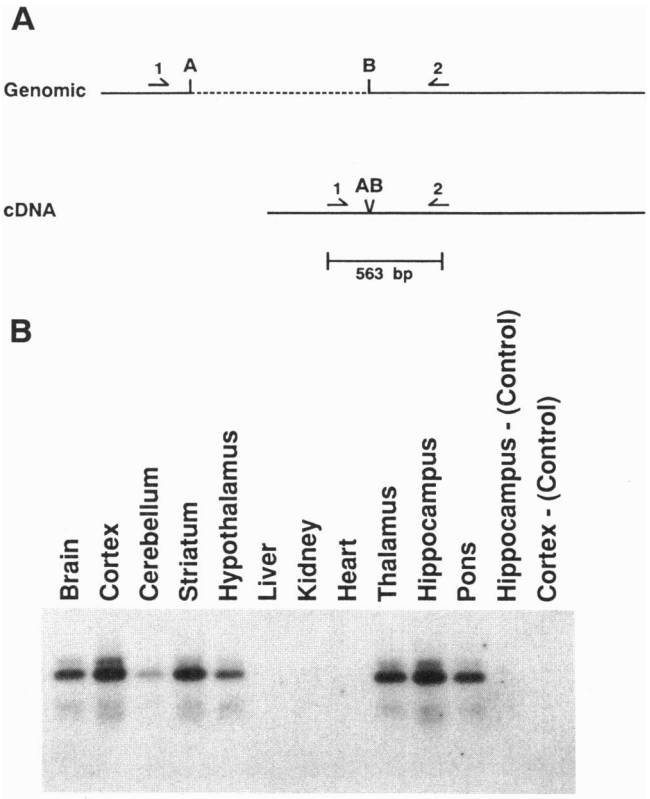

FIG. 3. PCR-based detection of MR77 mRNA expression. (*A*) Point B corresponds to nucleotide 97 of the MR77 genomic clone (Fig. 1). The distance between oligonucleotides 1 and 2 plus their lengths is 563 bp on the cDNA clone. The distance between point A and B on the genomic clone is not known. (*B*) The regional distribution of MR77 mRNA expression. cDNAs from the tissues listed were used as templates for the PCR reaction in *A*. The major band (middle band) migrates at a size approximating 570 bp. The blot was probed with an ^{32}P-labeled oligonucleotide corresponding to nucleotides 257–278 of the genomic clone. Control lanes 12 and 13 contained mRNA templates that had not been reverse-transcribed.

MR77 cDNA. The MR77 PCR product was detected in approximately the following abundance: cortex = striatum = hippocampus > thalamus = pons > hypothalamus > cerebellum. This distribution is consistent with known central nervous system terminal fields for serotonergic pathways. The abundance of MR77 mRNA in the cerebral cortex was estimated to be approximately 0.0001% of total mRNA, based on the comparison of the PCR product formed from cortex cDNA template to the product formed from known amounts of cloned MR77 DNA template. No PCR product was detected with liver, kidney, or heart cDNA templates. To show that the PCR products were not the result of amplification of genomic DNA, the same hippocampus and cortex mRNAs used to make cDNA template were additionally used as templates in PCR. These reactions did not yield any products (lanes 12 and 13, Fig. 3*B*).

Expression and Pharmacological Analysis of MR77. To determine the pharmacological profile of this receptor, we subcloned the gene into a eukaryotic expression vector (pCMV4) and transfected CosM6 cells. Broken cell preparations from transfected cells exhibited saturable ^{125}I-LSD binding that was competitively antagonized by 5-HT but not by either of the two catecholamines dopamine and norepinephrine. Saturation isotherm binding experiments demonstrated that ^{125}I-LSD binds with high affinity (K_d = 21.2 nM ± 5) to an apparent single class of noninteracting binding sites.

Table 1 shows the IC_{50} values for competition by a variety of selective and nonselective 5-HT ligands for ^{125}I-LSD binding to MR77 and the reported 5-HT$_{1E}$ receptor. MR77 demonstrated a high affinity for 5-HT, much like members of the 5-HT$_1$ subfamily. As predicted by the primary sequence analysis, MR77 exhibited a 5-HT$_{1D}$-like pharmacology in that it had affinity for sumatriptan but not 8-hydroxy-2(di-*n*-propylamino)tetralin. However, MR77 did not bind the mixed 5-HT$_{1A/1D}$ agonist 5-CT. MR77 also exhibited high affinity for the ergot alkaloids ergotamine and methysergide. ^{125}I-LSD binding to MR77 was relatively unaffected by the 5-HT$_{1C/2}$-selective ligands methiothepin and mesulergine. Thus, MR77 has a unique pharmacological profile, and its affinity for 5-HT classifies it as a member of the 5-HT$_1$ subfamily.

Table 1. Competition studies for ^{125}I-LSD-labeled sites on rat MR77 vs. 5-HT$_{1E}$ receptor affinity constants

Ligand	MR77 IC_{50}, nM ± SE	5-HT$_{1E}$ K_i, nM
Methysergide	14 ± 2	300
Ergotamine	55 ± 7	600
Sumatriptan	67 ± 3	2300
5-HT	70 ± 3	7
Metergoline	535 ± 43	1100
Yohimbine	590 ± 31	—
5-CT	2593 ± 662	7100
8-OH-DPAT	>1000	—
SKF 83566	>1000	—
Mesulergine	>1000	—
Dopamine	>1000	—
Norepinephrine	>1000	—
Methiothepin	>1000	200

CosM6 cells were transfected with an expression vector containing MR77 cDNA. Cell membranes were labeled with ^{125}I-LSD, which was competitively antagonized by a variety of ligands. IC_{50} values represent the concentrations of ligands (nM ± SEM) at which 50% of the bound ^{125}I-LSD could be displaced. 5-HT$_{1E}$ values are from McAllister *et al.* (9). 5-CT, 5-carboxyamidotryptamine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin.

The insensitivity of MR77 to 5-CT was similar to that observed for the reported human 5-HT_{1E} receptor (12, 13). Leonhardt and coworkers (13) demonstrated that the putative 5-HT_{1E} receptor had high affinity for 5-HT, ergotamine, and methysergide but not 5-CT. Although the reported K_i values for ligand binding at the 5-HT_{1E} receptor were slightly different than those for MR77, the general trend was similar. McAllister *et al.* (9) have recently cloned DNA for a human receptor that was identical to the *S31* gene and have reported that the pharmacology of the receptor matches exactly that for the putative 5-HT_{1E} receptor (13). Table 1 lists the K_i values for competition binding vs. tritiated 5-HT in cells transfected with the S31 receptor gene. The 5-HT_{1E} receptor is insensitive to sumatriptan, whereas MR77 displays significant affinity for this ligand. In addition, the ergot alkaloids methysergide and ergotamine display lower affinity for the 5-HT_{1E} receptor than for MR77. It should be noted that our binding assays used ^{125}I-LSD, whereas the 5-HT_{1E} studies used tritiated 5-HT. These different agents may result in the variations between affinity constants determined for the ligands tested.

Inhibition of Adenylate Cyclase. The primary structure of MR77 suggested that this receptor may be coupled to the inhibition of adenylate cyclase through an inhibitory G-protein (G_i)-like protein. This assumption was based on similarities among G_i-coupled receptors, which tend to have longer intracellular loops between transmembrane regions five and six as well as shorter C-terminal tails compared with stimulatory G-protein (G_s)-coupled receptors. We tested whether MR77 could mediate the inhibition of adenylate cyclase induced by serotonin. In cells cotransfected with MR77 and the hamster β_2-adrenergic receptor, 10 μM 5-HT elicited a 32 $\pm$ 6% (n = 3) decrease of isoproterenol-stimulated cAMP accumulation. Cells cotransfected with the rat 5-HT_{1A} receptor, known to couple to G_i, and the hamster β_2-adrenergic receptor exhibited a 53 $\pm$ 9% (n = 3) reduction in cAMP accumulation in response to 10 μM 5-HT. Cells transfected with the hamster β_2-adrenergic receptor alone showed almost no inhibition (8 $\pm$ 6%) in response to 10 μM 5-HT. These results show that MR77 is capable of coupling in an inhibitory manner to adenylate cyclase, presumably through a G_i-like mechanism.

CONCLUSION

The data presented demonstrate that the MR77 gene encodes a 5-HT receptor protein that is expressed predominantly in the central nervous system. The mRNA distribution pattern in the brain is consistent with terminal fields for some serotonergic neurons. MR77 couples to the inhibition of adenylate cyclase and has a binding profile similar to that for the 5-HT_{1D} receptor with the marked exception in affinity for 5-CT. Interestingly, sumatriptan and ergotamine, both of which exhibit high affinity for MR77, have been shown to have clinical efficacy for the treatment of migraine headaches (for review, see ref. 27). This raises the possibility that the action of these drugs may be mediated by more than one subtype of 5-HT receptors.

Lately, the identification of receptor genes has outpaced traditional pharmacological identification. Therefore, it has become increasingly difficult to classify receptor gene products on a basis other than amino acid sequence. The pharmacological profile of the MR77 receptor protein is similar to the previously identified 5-HT_{1E} receptor with regard to the apparent insensitivity to the ligand 5-CT but differs with regard to the affinity for sumatriptan. Given that the sequence of MR77 is most similar to 5-HT_{1E}, we propose that the MR77 gene be tentatively identified as another member of the 5-HT_{1E} receptor subgroup.

Note Added in Proof. The mouse homolog of this 5-HT_{1E}-like receptor has been reported (28).

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