Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5517–5521, June 1992
Neurobiology

Molecular cloning of a serotonin receptor from human brain (5HT1E): A fifth 5HT1-like subtype

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Contributed by L. L. Iversen, February 24, 1992

ABSTRACT **Degenerate primers, suitable for polymerase chain reaction studies and based on the conserved structure of G protein-coupled receptors, were used to isolate cDNA clones encoding putative G protein-coupled receptors from a human hippocampal cDNA library. One clone isolated by this approach (AC1) encoded a putative receptor with 39% amino acid sequence identity to the serotonin 5HT1A receptor and 47% identity to the 5HT1D receptor. When expressed transiently in the human embryonic kidney cell line 293, AC1 cDNA-encoded receptor displayed high affinity (K_d = 15 nM) and saturability for [^{3}H]serotonin, suggesting that AC1 encodes a 5HT1-like receptor. However, 5-carboxamidotryptamine demonstrated low affinity (pK_i = 5.15) compared with serotonin (pK_i = 8.14), consistent with the observed binding of the putative 5HT1E receptor. The excellent correlation observed between the pharmacology of the expressed receptor encoded by AC1 and the human brain 5HT1E binding site confirms that AC1 encodes a 5HT1E receptor and establishes a fifth 5HT1-like receptor subtype.**

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter thought to play a role in various cognitive and behavioral functions including feeding, sleep, pain, depression, and learning (reviewed in ref. 1). Serotonin receptors have been divided into four classes designated 5HT1-like, 5HT2, 5HT3 (2), and 5HT4 (3), depending on their ligand binding and effector-coupling properties. The 5HT1 receptor family can be further subdivided into four subtypes, 5HT1A–5HT1D (4). A putative 5HT1E subtype has been described (5). 5HT1-like receptors demonstrate high-affinity binding of 5-HT (K_i < 100 nM) and functionally couple via guanine nucleotide-binding proteins (G proteins). This plethora of 5-HT receptors, particularly the 5-HT1-like subtypes, makes it difficult to determine specific functional and behavioral correlates for each member of this family. Some receptor-selective agonist compounds do exist, but as yet there are no selective antagonists available for 5HT1-like receptors. The availability of cloned 5HT1 subtypes, expressed in cell lines as pure populations, should greatly facilitate the development of subtype-specific drugs.

In this work we have exploited the fact that the G protein-coupled receptor gene family members share similar structural features. The most obvious common feature is the presence of seven putative transmembrane regions (6, 7). Several groups have now used this structural and sequence conservation to make degenerate oligonucleotide primers corresponding to the most highly conserved transmembrane regions (8, 9). Such primers can then be used to amplify cDNAs from suitable sources and identify novel G protein-coupled receptors. We have synthesized degenerate primers devised from transmembrane regions III and VI of the 5HT1A receptor (10), substance K receptor (11), and the α_2-, β_1-, and β_2-adrenergic receptors (12–14). Here we describe the cloning and characterization of a 5HT1E receptor with these techniques.†

MATERIALS AND METHODS

cDNA Cloning. Two degenerate oligonucleotides [5′-GGAATTC(C/A)TG(T/A)(G/C)TG(C/T)CAT(T/C)G(G/C)NNT(G/T)GAC(C/A)G(C/G)TAC-3′ and 5′-AAAGCTTA(T/G)G(A/T)(A/G)G(A/T)AGGGCAGCCAGCAGA(G/C/T)G(G/A)(T/C)(G/A)AA-3′] were synthesized on an Applied Biosystems 380B instrument. The two primers were employed in a polymerase chain reaction (92°C, 1 min; 55°C, 2 min; 72°C, 4 min; 30 cycles) using cDNA synthesized from monkey cortex poly(A)$^+$ RNA as a template. Reaction products (≈500 base pairs long) were restriction digested (*Eco*RI/*Hin*dIII) and subcloned into the pBluescript II SK(−) vector (Stratagene) for subsequent analysis. Following preliminary DNA sequencing (15), one such cloned product (M3) appeared likely to encode a G protein-coupled receptor. M3 insert cDNA was radiolabeled (16) and used to screen a human hippocampal cDNA library (Stratagene) constructed in λZAP. The ^{32}P-labeled insert was hybridized [5× SSPE (1× is 0.18 M NaCl/0.01 M sodium phosphate, pH 7.4/1 mM EDTA)/30% formamide/5× Denhardt's solution/0.5% SDS plus denatured salmon testes DNA at 250 μg/ml] at 42°C overnight to filters containing ≈5 × 10^5 plaques from the phage library. Filters were subsequently washed thrice in 5× SSPE/0.1% SDS for 20 min and exposed to autoradiographic film. Of four positive clones isolated, one (AC1) was fully sequenced (15) and further characterized.

Transient and Stable Expression of AC1. A *Xho* I–*Not* I restriction fragment of clone AC1 containing the entire cDNA insert (≈2 kilobases) was inserted between *Xho* I and *Not* I sites of the expression vector pCDM8 (17). This pCDM8-AC1 plasmid was used to transfect (18) the human embryonic kidney (HEK) cell line 293 and give transient expression of the AC1-encoded receptor. Binding assays (see below) were performed on crude membrane suspensions prepared from cells 48 hr after transfection.

Stably transfected cell lines were obtained by cotransfecting the AC1-containing pCDM8 vector with pcDNAneo (Invitrogen) into HEK 293 cells by the calcium phosphate method (19). Transformed cells were selected for their resistance to the antibiotic G418 (1 mg/ml) and assayed for their ability to bind [^{3}H]5-HT. Cells that demonstrated binding were then subjected to single-cell dilution cloning and one clone (IV-1) was used for subsequent studies.

[^{3}H]5-HT Binding. Radioligand binding assays with [^{3}H]5-HT (2–3 nM) (≈10 Ci/mmol; New England Nuclear; 1 Ci = 37 GBq) were performed on crude membrane preparations

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Abbreviations: 5-HT, 5-hydroxytryptamine; 5-CT, 5-carboxamidotryptamine; HEK, human embryonic kidney.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M91467).

from HEK 293 cells. Nonspecific binding was determined in the presence of 10 μM 5-HT and typically represented 5–10% of total binding. Incubations were terminated by vacuum filtration. Competition assay data are average values of duplicate determinations from representative experiments and were modeled by nonlinear regression analysis by computer using a one-site model (20). [^{3}H]5-HT binding studies on the 5HT1E binding site in human frontal cortex were carried out in the presence of 100 nM 5-carboxamidotryptamine (5-CT) and 100 nM mesulergine (5). Nonspecific binding represented 45–55% of total binding.

Adenylate Cyclase Activity. The formation of [^{32}P]cAMP from [^{32}P]ATP by crude membrane preparations was monitored essentially as described (21). The incubation medium (50 μl per tube) was 50 mM Tris·HCl (pH 7.6 at room temperature) containing 100 mM NaCl, 0.03 mM GTP, 0.05 mM cAMP, 0.5 mM ATP, 1 mM dithiothreitol, 5 mM $MgCl_2$, 3.5 mM creatine phosphate, 0.2 mg of creatine kinase per ml, 1 μCi of [^{32}P]ATP, 1 nCi of [^{3}H]cAMP, 10 μM forskolin, 0.5 mM EGTA, 0.5 mM 3-isobutyl-1-methylxanthine, and test drugs or vehicle (0.1% ascorbate). The reaction was initiated by the addition of membrane (5 μg of protein per tube). After 14 min at 30°C, the reaction was terminated by addition of 100 μl of 2% SDS/45 mM ATP/1.3 mM cAMP. The [^{32}P]ATP and [^{32}P]cAMP were separated through the double column system [Dowex exchange resin AG50Wx4 (Bio-Rad) and neutral alumina] (22). Dose–response curves were plotted and agonist potencies were calculated as pEC_{50} values ($-\log EC_{50}$).

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AGT GAG AAA CCT TCG AGG CTA CAT AGT TTT CAG CCA AAG GAA AAT AAC CAA CAG   -34

CTT CTC CAC AGT GTA GAC TGA AAC AAG GGA AAC ATG AAC ATC ACA AAC TGT ACC    21
                                           MET Asn Ile Thr Asn Cys Thr     7
                                               ▲       ▲

ACA GAG GCC AGC ATG GCT ATA AGA CCC AAG ACC ATC ACT GAG AAG ATG CTC ATT    75
Thr Glu Ala Ser MET Ala Ile Arg Pro Lys Thr Ile Thr Glu Lys MET Leu Ile    25

TGC ATG ACT CTG GTG GTC ATC ACC ACC CTC ACC ACG TTG CTG AAC TTG GCT GTG   129
Cys MET Thr Leu Val Val Ile Thr Thr Leu Thr Thr Leu Leu Asn Leu Ala Val    43

ATC ATG GCT ATT GGC ACC ACC AAG AAG CTC CAC CAG CCT GCC AAC TAC CTA ATC   183
Ile MET Ala Ile Gly Thr Thr Lys Lys Leu His Gln Pro Ala Asn Tyr Leu Ile    61

TGT TCT CTG GCC GTG ACG GAC CTC CTG GTG GCA GTG CTC GTC ATG CCC CTG AGC   237
Cys Ser Leu Ala Val Thr Asp Leu Leu Val Ala Val Leu Val MET Pro Leu Ser    79

ATC ATC TAC ATT GTC ATG GAT CGC TGG AAG CTT GGG TAC TTC CTC TGT GAG GTG   291
Ile Ile Tyr Ile Val MET Asp Arg Trp Lys Leu Gly Tyr Phe Leu Cys Glu Val    97

TGG CTG AGT GTG GAC ATG ACC TGC TGC ACC TGC TCC ATC CTC CAC CTC TGT GTC   345
Trp Leu Ser Val Asp MET Thr Cys Cys Thr Cys Ser Ile Leu His Leu Cys Val   115

ATT GCC CTG GAC AGG TAC TGG GCC ATC ACC AAT GCT ATT GAA TAC GCC AGG AAG   399
Ile Ala Leu Asp Arg Tyr Trp Ala Ile Thr Asn Ala Ile Glu Tyr Ala Arg Lys   133

AGG ACG GCC AAG AGG GCC GCG CTG ATG ATC CTT ACC GTC TGG ACC ATC TCC ATT   453
Arg Thr Ala Lys Arg Ala Ala Leu MET Ile Leu Thr Val Trp Thr Ile Ser Ile   151

TTC ATC TCC ATG CCC CCT CTG TTC TGG AGA AGC CAC CGC CGC CTA AGC CCT CCC   507
Phe Ile Ser MET Pro Pro Leu Phe Trp Arg Ser His Arg Arg Leu Ser Pro Pro   169

CCT AGT CAG TGC ACC ATC CAG CAC GAC CAT GTT ATC TAC ACC ATT TAC TCC ACG   561
Pro Ser Gln Cys Thr Ile Gln His Asp His Val Ile Tyr Thr Ile Tyr Ser Thr   187

CTG GGT GCG TTT TAT ATC CCC TTG ACT TTG ATA CTG ATT CTC TAT TAC CGG ATT   615
Leu Gly Ala Phe Tyr Ile Pro Leu Thr Leu Ile Leu Ile Leu Tyr Tyr Arg Ile   205

TAC CAC GCG GCC AAG AGC CTT TAC CAG AAA AGG GGA TCA AGT CGG CAC TTA AGC   669
Tyr His Ala Ala Lys Ser Leu Tyr Gln Lys Arg Gly Ser Ser Arg His Leu Ser   223

AAC AGA AGC ACA GAT AGC CAG AAT TCT TTT GCA AGT TGT AAA CTT ACA CAG ACT   723
Asn Arg Ser Thr Asp Ser Gln Asn Ser Phe Ala Ser Cys Lys Leu Thr Gln Thr   241

TTC TGT GTG TCT GAC TTC TCC ACC TCA GAC CCT ACC ACA GAG TTT GAA AAG TTC   777
Phe Cys Val Ser Asp Phe Ser Thr Ser Asp Pro Thr Thr Glu Phe Glu Lys Phe   259

CAT GCC TCC ATC AGG ATC CCC CCC TTC GAC AAT GAT CTA GAT CAC CCA GGA GAA   831
His Ala Ser Ile Arg Ile Pro Pro Phe Asp Asn Asp Leu Asp His Pro Gly Glu   277

CGT CAG CAG ATC TCT AGC ACC AGG GAA CGG AAG GCA GCA CGC ATC CTG GGG CTG   885
Arg Gln Gln Ile Ser Ser Thr Arg Glu Arg Lys Ala Ala Arg Ile Leu Gly Leu   295

ATT CTG GGT GCA TTC ATT TTA TCC TGG CTG CCA TTT TTC ATC AAA GAG TTG ATT   939
Ile Leu Gly Ala Phe Ile Leu Ser Trp Leu Pro Phe Phe Ile Lys Glu Leu Ile   313

GTG GGT CTG AGC ATC TAC ACC GTG TCC TCG GAA GTG GCC GAC TTT CTG ACG TGG   993
Val Gly Leu Ser Ile Tyr Thr Val Ser Ser Glu Val Ala Asp Phe Leu Thr Trp   331

CTC GGT TAT GTG AAT TCT CTG ATC AAC CCT CTG CTC TAT ACG AGT TTT AAT GAA  1047
Leu Gly Tyr Val Asn Ser Leu Ile Asn Pro Leu Leu Tyr Thr Ser Phe Asn Glu   349

GAC TTT AAG CTG GCT TTT AAA AAG CTC ATT AGA TGC CGA GAG CAT ACT TAG ACT  1101
Asp Phe Lys Leu Ala Phe Lys Lys Leu Ile Arg Cys Arg Glu His Thr          365

GTA AAA AGC TAA AAG GCA CGA CTT TTT CCA GAG CCT CAT GAG TGG ATG GGG GTA  1155
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FIG. 1. Nucleotide and deduced amino acid sequence of AC1 cDNA. Nucleotide numbers are given above amino acid numbers. The first nucleotide of the initiating methionine codon is designated +1. This initiation site was chosen because it fits best the Kozak translation initiation consensus sequence (24). Potential transmembrane domains are underlined. Two potential N-linked glycosylation sites (▲) are indicated.

RESULTS AND DISCUSSION

Degenerate oligonucleotide primers corresponding to putative G protein-coupled receptor transmembrane domains III and VI were synthesized and used in a polymerase chain reaction (23) with monkey cortex cDNA as a template. Reaction products of ≈500 base pairs were observed upon agarose gel electrophoresis and these were subcloned into pBluescript. Sequencing of individual subclones suggested that one of these (M3) was likely to encode a G protein-coupled receptor (data not shown). M3 insert cDNA was radiolabeled (16) and used to screen a human hippocampal cDNA library. Several positive clones were identified. Characterization of one such clone (AC1) is described in this work. Clone AC1 contained an insert of ≈2 kilobases and the longest open reading frame encoded a 365-amino acid polypeptide (Fig. 1). Hydrophobicity analysis of AC1 demonstrated the seven putative transmembrane domains (data not shown) characteristic of G protein-coupled receptors. Comparison of the deduced amino acid sequence of AC1 with the sequence of other G protein-linked receptors revealed that it was most similar with the putative dog 5HT1D receptor RDC4 (8) and the human 5HT1A receptor (10, 25). In fact, during the course of these studies, the probable human analogue of RDC4 was cloned and shown to encode a human 5HT1D receptor (26). Therefore, cDNA AC1 encodes a putative receptor with considerable similarity to the 5HT1D (47% identity) and 5HT1A (39% identity) receptors (Fig. 2), suggesting that AC1 encodes a 5HT1-like receptor (2). This putative receptor is, however, distinct from the two other subtypes of 5-HT1-like receptor so far identified [5HT1B (27) and 5HT1C (28)] and the 5HT2 receptor (29), with which it displays only low homology (data not shown).

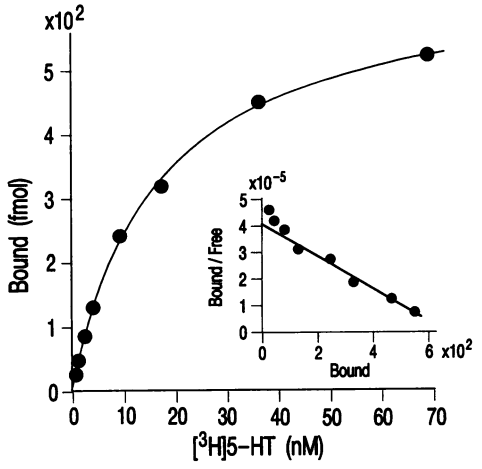

FIG. 3. Saturation and Scatchard (*Inset*) analyses were carried out by incubating cell membranes from AC1-transfected HEK 293 cells with various concentrations of [³H]5-HT. A representative experiment is shown here. K_d = 15 ± 0.86 nM (mean ± SEM, n = 3). B_{max} values varied from 15 to 40 pmol/mg of protein in different experiments.

The 5HT1-like nature of the receptor encoded by AC1 cDNA was confirmed by expression studies in which AC1 cDNA was subcloned into the eukaryotic expression vector pCDM8 (14) and transiently expressed in HEK 293 cells. Membranes prepared from AC1-transfected HEK 293 cells were found to express specific, high-affinity [³H]5-HT binding sites, whereas membranes from mock-transfected or wild-type cells exhibited no specific binding (data not shown). In AC1-transfected HEK 293 cells, nonlinear regression analysis of [³H]5-HT saturation isotherms (Fig. 3) was consistent with the

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                                                                I
   AC1      1 MnitN-----cTTEASmairpkTiTe----------KmlicmtLvVITtlTtLlN
5-HT1D      1 MspLNqsaeglpnEASnrslnaTeTseawDprtlqalKiSLavvLsVITlaTVLsN
5-HT1A      1 MdvLspgqgNnTTsppapfetggnTtgisDvtvsyqvitSL--lLgtlifcaVLgN

                                                II
   AC1     41 laVimAIgtTkKLHqPANYLIcSLAVTDLLVaVLVMPlSIiYiVmdrWkLGyfLCe
5-HT1D     57 AfVlttIlLTRKLHtPANYLIGSLAtTDLLVSiLVMPiSIaYtithtWnfGQiLCD
5-HT1A     55 AcVvaAIaLeRsLqnvANYLIGSLAVTDLmVSVLVlPmaalYqVlnkWtLGQvtCD

                     III                                        IV
   AC1     97 vWLSvDmTCCTcSILHLCVIALDRYWAITnAIEYarKRTAkRAAlMIltVWtISIf
5-HT1D    113 iWLSsDiTCCTaSILHLCVIALDRYWAITDAlEYskrRTAghAAtMIaiVWaISIc
5-HT1A    111 lfialDvlCCTsSILHLCaIALDRYWAITDpIdYvnKRT-pRpralIsltWlIgfl

                                                      V
   AC1    153 ISmPPLFWRshrrlspppSqCTIqhDHviYTIYSTlGAFYIPLtLiLILYyRIYhA
5-HT1D    169 ISIPPLFWR-qakaqeemSdClvntsqisYTIYSTcGAFYIPsvLliILYGRIYRA
5-HT1A    166 ISIPPilgwrtpedrsdpdaCTIskDH-gYTIYSTfGAFYIPLlLmLvLYGRIfRA

   AC1    209 AkslyqKrgSsrhlsnrsTdsqnSfAscklTqtfcv----------
5-HT1D    224 ARnRIlnppSlygKrfttahlitgsAgsslc----------
5-HT1A    221 ARfRIrKtvkkveKtgadTrhgaSpAapqpkksvngesgsrnwrlgveskaggalca

   AC1    245 ----------SdfStsdpttefekfhasirippfdndlDhpgERq---------
5-HT1D    255 ----------SlnSslHeghshsagsPLffnhvkiklADsalERK---------
5-HT1A    277 ngavrqgddgaalevievHrvgnskehlPLpseagptpcApasfERKnernaeakr

                                  VI
   AC1    280 qISstRERKAarILGlILGAFILsWLPFFIkeLivgls--iytvssevaDFlTWLG
5-HT1D    290 rISaARERKAtKILGIILGAFIiCWLPFFvVsLVLPiCrdSCwihpaLfDFfTWLG
5-HT1A    333 kmalARERKtvKtLGIImGtFILCWLPFFIVaLVLPfCesSChmptlLgaiinWLG

                VII
   AC1    334 YvNSLINPllYTsFNEDFklAFKKlIrCReht
5-HT1D    346 YlNSLINPiIYTvFNEeFrqAFqKIvpfRkas
5-HT1A    389 YsNSLlNPvIYayFNkDFqnAFKKIIkCnfcrq
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FIG. 2. Alignment of the deduced AC1 amino acid sequence with those of the human 5HT1D (26) and 5HT1A (25) receptors. Amino acids conserved between subtypes are shown by uppercase letters. Gaps in the sequences (−) are indicated, and putative transmembrane regions are overlined.

presence of a single class of binding sites with a K_d of 15 ± 0.86 nM (mean ± SEM, n = 3). B_{max} values in the various transfections ranged from 15 to 40 pmol/mg of protein.

Competition studies were performed to examine the pharmacological characteristics of the AC1 encoded receptors. First, specific [^{3}H]5-HT binding was investigated in the presence of saturating concentrations of drugs that bind to the 5HT1A and 5HT1B (cyanopindolol), 5HT1C (mesulergine), 5HT1D (5-CT), 5HT2 (ketanserin), and 5HT3 and 5HT4 (ICS 205,930) receptors as well as drugs that would block the 5-HT uptake site (imipramine, paroxetine) and the 5-HT binding site on storage granules within the nerve terminal (reserpine). None of these drugs abolished specific [^{3}H]5-HT binding (Fig. 4), suggesting that AC1 encodes a novel 5HT1-like receptor. High-affinity 5-HT binding is characteristic of 5HT1-like receptor subtypes but not 5-HT2 or 5-HT3 receptors. These data, together with the homology of AC1 with 5HT1A and 5HT1D receptors, strongly suggest that AC1 encodes a 5HT1-like receptor subtype. This was confirmed by further radioligand binding studies. [^{3}H]5-HT binding to membranes from AC1-transfected HEK 293 cells was displaced by a range of drugs yielding data consistent with the presence of a single class of binding site (Table 1). A typical experiment is shown in Fig. 5. The striking feature of these studies is the very low affinity of 5-CT (pK_i = 5.15 ± 0.03, mean ± SEM, n = 3) for AC1-encoded receptors. This pharmacological profile is not consistent with any known 5HT receptor subtype but it is consistent with a [^{3}H]5-HT binding site putatively identified as a 5HT1E receptor by Leonhardt *et al.* (5). Using binding studies on human brain membranes, they identified a high-affinity [^{3}H]5-HT binding site in cortex with low affinity for 5-CT and proposed this site as a putative 5HT1E receptor. Assays were carried out in the presence of 100 nM 5-CT and 100 nM mesulergine to block the other 5HT1-like receptors. We have also examined the pharmacological profile of this site on human cortical membranes in parallel with membranes from HEK 293 cells transiently and stably transfected with AC1 cDNA (Table 1). Comparison of the pK_i values of both cloned and native sites yields a correlation coefficient of r = 0.86, $P < 0.02$ (Table 1), strongly suggesting that AC1 encodes a human 5HT1E receptor.

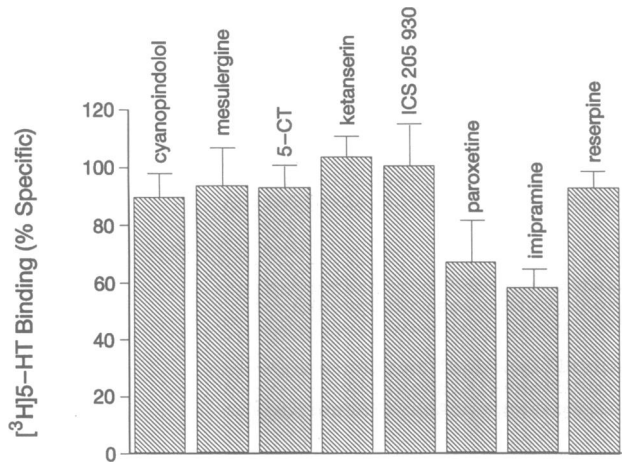

FIG. 4. Competition of specific [^{3}H]5-HT binding to membranes from AC1-transfected HEK 293 cells. 5-HT (10 μM) was used to define nonspecific binding. Data are presented as percentages of control specific [^{3}H]5-HT binding and represent the means ± SEM of three separate experiments performed in triplicate. [^{3}H]5-HT (2 nM) binding assays were carried out in the presence of cyanopindolol (100 nM), mesulergine (100 nM), 5-CT (100 nM), ketanserin (10 μM), ICS 205,930 (10 μM), paroxetine (10 μM), imipramine (10 μM), and reserpine (50 μM). Details of the selectivity of these drugs are given in the text.

Table 1. Competition for [^{3}H]5-HT binding sites

Drug	pK_i (mean ± SEM, $n \geq 3$)		
	Transient	Stable	Frontal cortex
5-HT	8.14 ± 0.02	8.21 ± 0.08	8.23 ± 0.19
5-CT	5.15 ± 0.03	5.48 ± 0.05	5.67 ± 0.09
Methysergide	6.49 ± 0.04	6.66 ± 0.02	6.76 ± 0.09
Sumatriptan	5.63 ± 0.02	5.68 ± 0.08	5.89 ± 0.05
Metergoline	5.95 ± 0.03	6.11 ± 0.05	6.37 ± 0.11
Methiothepin	6.68 ± 0.02	6.92 ± 0.16	5.81 ± 0.09
Ergotamine	6.24 ± 0.02	6.27 ± 0.09	6.10 ± 0.19

Values indicate affinities of selected drugs for [^{3}H]5-HT (2–3 nM) binding sites on membranes prepared from HEK 293 cells, either transiently (transient) or stably (stable) transfected with AC1 cDNA, or from human frontal cortex. n is the number of independent determinations. All curves were best fit to a single class of binding sites by nonlinear regression analysis. pK_d values for [^{3}H]5-HT binding were 7.82 ± 0.02 (mean ± SEM, n = 3), 8.15 ± 0.09 (n = 4), and 8.17 (n = 2) for transiently transfected cell membranes, human frontal cortex membranes, and stably transfected cell membranes, respectively. B_{max} values in transiently transfected cells ranged from 15 to 40 pmol/mg of protein compared with 170 fmol/mg of protein in frontal cortex and 8.4 pmol/mg of protein in stably transfected cells.

Although the predicted amino acid sequence of AC1 is characteristic of G protein-coupled receptors, guanine nucleotide sensitivity of [^{3}H]5-HT receptor binding could not be demonstrated in transiently or stably transfected HEK 293 cells (data not shown). However, a functional response of adenylate cyclase activity was observed in HEK 293 cells stably transfected with AC1 cDNA (Fig. 6). In agreement with the observed pharmacology (i.e., high affinity for 5-HT and low affinity for 5-CT), 5-HT was ≈500-fold more potent than 5-CT in producing an ≈20% decrease in forskolin-stimulated adenylate cyclase activity (pEC_{50} for 5-HT, 6.97 ± 0.14; pEC_{50} for 5-CT, 4.26 ± 0.11, mean ± SEM, n = 3). The lack of guanine nucleotide sensitivity of [^{3}H]5-HT binding and the relatively small magnitude of the adenylate cyclase response would be explained if HEK 293 cells possess only low levels of the appropriate G protein required to couple to the receptor. However, these results demonstrated that the AC1 receptor is functionally coupled and, like the 5HT1A, 5HT1B, and 5HT1D receptors (30), can mediate the inhibition of adenylate cyclase activity.

These data strongly suggest that AC1 encodes a functionally coupled human 5HT1E receptor and confirm the existence of a fifth 5HT1-like receptor subtype. It is unclear what the functional significance of these subtypes is, but agents

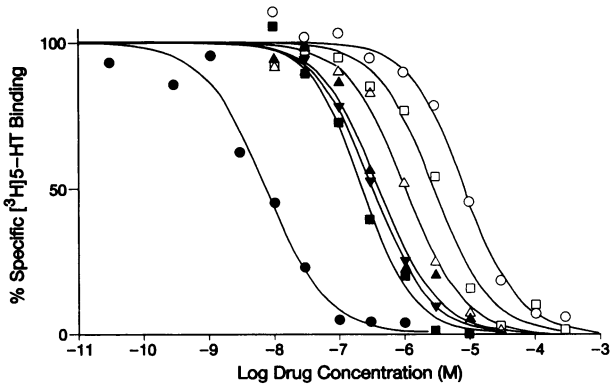

FIG. 5. Typical profiles of the ability of various compounds to compete with [^{3}H]5-HT (2 nM) for binding to membranes from AC1-transfected HEK 293 cells. Mean pK_i values are given in Table 1. The compounds used were 5-HT (●), methiothepin (■), methysergide (▼), ergotamine (▲), metergoline (△), sumatriptan (□), and 5-CT (○).

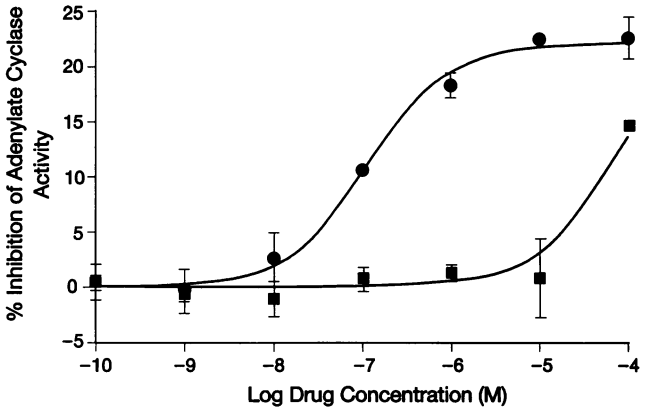

FIG. 6. Inhibition of forskolin-stimulated adenylate cyclase activity by 5-HT (●) and 5-CT (■). Formation of [^{32}P]cAMP from [^{32}P]ATP was measured (22) in a crude membrane preparation from a stably transfected cell line (HEK clone IV-1). Results shown are the means ± SEM of three experiments.

that interact with 5HT1-like receptors have been used in the treatment of various neuropsychiatric disorders (reviewed in refs. 31 and 32). For example, selective 5HT1A agents have been developed as potential anxiolytics and have also been shown to possess antidepressant properties in various animal models. Sumatriptan, a 5HT1D-selective receptor agonist, has been reported to be an effective treatment for migraine. The existence of molecular probes for these 5HT1-like subtypes will enable the regional distribution of the receptors to be determined, thus yielding some insight into their function. This, and the availability of stable cell lines expressing the various human receptors, will facilitate studies of their regulatory properties and should greatly assist in the identification of subtype-selective compounds to investigate the functions of these receptors *in vivo*. In particular, for the previously uncharacterized 5HT1E receptor, the low affinity for 5-CT suggests that the binding site of this subtype differs significantly from those of other 5HT1-like receptors. These further studies should determine whether the 5HT1E receptor will be an important drug target in the treatment of neuropsychiatric disorders.

Note Added in Proof. After submission of this manuscript we became aware of a paper by Levy *et al.* (33) describing the cloning of a novel serotonin receptor gene (S31). The sequence of this gene is identical to that of AC1 reported here. However, the receptor binding pharmacology of S31 was not determined and, therefore, S31 was not identified as a 5HT1E receptor.

1. Wilkinson, L. O. & Dourish, C. T. (1991) in *Serotonin Receptor Subtypes: Basic and Clinical Aspects*, ed. Peroutka, S. J. (Wiley-Liss, New York), pp. 147–210.
2. Bradley, P. B., Engel, G., Feniuk, W., Fozard, J. R., Humphrey, P. P. A., Middlemiss, D. N., Mylecharone, E. J., Richardson, B. P. & Saxena, P. R. (1986) *Neuropharmacology* **25**, 563–576.
3. Dumuis, A., Sebben, M. & Bockaert, J. (1988) *Mol. Pharmacol.* **34**, 880–887.
4. Frazer, A., Maayani, S. & Wolfe, B. B. (1990) *Annu. Rev. Pharmacol. Toxicol.* **30**, 307–348.
5. Leonhardt, S., Herrick-Davis, K. & Titeler, M. (1989) *J. Neurochem.* **53**, 465–471.
6. Dixon, R., Kobilka, B., Strader, D., Benovic, J., Dohlman, H., Frielle, T., Bolanowski, M., Bennett, C., Rands, E., Diehl, R., Mumford, R., Slater, E., Sigal, I., Caron, M., Lefkowitz, R. & Strader, C. (1986) *Nature (London)* **321**, 75–79.
7. O'Dowd, B. F., Lefkowitz, R. J. & Caron, M. G. (1989) *Annu. Rev. Neurosci.* **12**, 67–83.
8. Libert, F. M., Parmentier, M., Lefort, A., Dinsart, C., Van Sande, J., Maenhaut, C., Simons, M.-J., Dumont, J. E. & Vassart, G. (1989) *Science* **244**, 569–572.
9. Frohman, M. A., Dush, M. K. & Martin, G. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8998–9002.
10. Kobilka, B. K., Frielle, T., Collins, S., Yang-Feng, T., Kobilka, T., Francke, U., Lefkowitz, R. J. & Caron, M. G. (1987) *Nature (London)* **329**, 75–79.
11. Masu, Y., Nakayama, K., Yamaki, H., Harada, Y., Kuno, M. & Nakanishi, S. (1987) *Nature (London)* **329**, 836–838.
12. Kobilka, B. K., Matsui, H., Kobilka, T. S., Yang-Feng, T. L., Francke, U., Caron, M. G., Lefkowitz, R. J. & Regan, J. W. (1987) *Science* **238**, 650–656.
13. Frielle, T., Collins, S., Daniel, K. W., Caron, M. G., Lefkowitz, R. J. & Kobilka, B. K. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7920–7924.
14. Kobilka, B. K., Dixon, R. A. F., Frielle, T., Dohlman, H. G., Bolanowski, M. A., Sigal, I. S., Yang-Feng, T. L., Francke, U., Caron, M. G. & Lefkowitz, R. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 46–50.
15. Sanger, F., Nicklen, S. & Coulson, A. R. (1979) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
16. Feinberg, A. & Vogelstein, B. (1984) *Anal. Biochem.* **137**, 266–267.
17. Seed, B. (1987) *Nature (London)* **329**, 840–842.
18. Chen, C. A. & Okayama, H. (1988) *BioTechniques* **6**, 632–637.
19. Cullen, B. R. (1987) *Methods Enzymol.* **152**, 684–704.
20. Munson, P. J. & Rodbard, D. (1980) *Anal. Biochem.* **107**, 220–239.
21. De Vivo, M. & Maayani, S. (1985) *Eur. J. Pharmacol.* **119**, 231–234.
22. Salomon, Y., Londos, C. & Rodbell, M. (1974) *Anal. Biochem.* **58**, 541–548.
23. Saiki, R. H., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
24. Kozak, M. (1986) *Cell* **42**, 283–292.
25. Fargin, A., Raymond, J. R., Lohse, M. J., Kobilka, B. K., Caron, M. G. & Lefkowitz, R. J. (1988) *Nature (London)* **335**, 358–360.
26. Hamblin, M. W. & Metcalf, M. A. (1991) *Mol. Pharmacol.* **40**, 143–148.
27. Voigt, M. V., Laurie, D. J., Seeburg, P. H. & Bach, A. (1991) *EMBO J.* **10**, 4017–4023.
28. Julius, D., McDermott, A. B., Axel, R. & Jessell, T. M. (1988) *Science* **241**, 558–564.
29. Julius, D., Huang, K. N., Livelli, T. J., Axel, R. & Jessell, T. M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 928–932.
30. Julius, D. (1991) *Annu. Rev. Neurosci.* **14**, 335–360.
31. Murphy, D. L. (1990) *Neuropsychopharmacology* **3**, 457–471.
32. Sleight, A. J., Pierce, P. A., Schmidt, A. W., Hekmatpanah, C. R. & Peroutka, S. J. (1991) in *Serotonin Receptor Subtypes: Basic and Clinical Aspects* (Wiley-Liss, New York), pp. 211–227.
33. Levy, F. O., Gudermann, T., Birnbaumer, M., Kaumann, A. J. & Birnbaumer, L. (1992) *FEBS Lett.* **296**, 201–206.