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Primary Structure and Functional Characterization of a Human 5-HT_{1D}-Type Serotonin Receptor

MARK W. HAMBLIN and MARK A. METCALF

Geriatric Research, Education, and Clinical Center, Seattle Veterans Affairs Medical Center, Seattle, Washington 98108 (M.W.H., M.A.M.), and the Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, Washington 98195 (M.W.H.)

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SUMMARY

We describe the nucleic acid sequence encoding a human 5-hydroxytryptamine$_{1D}$ (5-HT_{1D}) serotonin receptor and some of the functional characteristics of the gene product. The receptor gene was isolated by hybridization to a probe based on a canine thyroid cDNA (called RDC4) previously isolated by others and believed to encode a heretofore undetermined member of the guanine nucleotide-binding protein (G protein)-linked receptor family. The human clone we isolated, called MA6A, contains an apparently intronless open reading frame encoding a 377-amino acid polypeptide with the seven hydrophobic domains characteristic of G protein-linked receptors. The MA6A deduced amino acid sequence is 88% identical to that for RDC4 and 43% identical to that for the human 5-HT_{1A} receptor. Expression of the human gene product in transfected cell lines results in the appearance of saturable high affinity 5-HT_{1D}-type [^{3}H]5-HT binding. The expressed receptor exhibits features indicative of coupling to G_i proteins, i.e., robust inhibition of forskolin-stimulated cAMP accumulation and formation of a pertussis toxin-sensitive high agonist affinity binding state. These findings may help clarify several ambiguities in the classification and action of serotonin receptor subtypes.

Serotonin (5-HT) mediates diverse effects in mammals through at least eight discrete receptor subtypes (1). The nucleic acid sequence of three G protein-linked mammalian serotonin receptors, the 5-HT_{1A} (2, 3), 5-HT_{1C} (4), and 5-HT_2 (5) receptors, has been described, as has that of a structurally related *Drosophila* serotonin receptor (6). One serotonin receptor subtype whose nucleic acid sequence has not yet been established is the 5-HT_{1D} receptor. First defined in radioligand binding studies, 5-HT_{1D} receptors are widely distributed in the central nervous system (7). Although some 5-HT_{1D}-type receptors serve as presynaptic terminal autoreceptors inhibiting serotonin release (8), others are likely present on nonserotonergic neurons (9). 5-HT_{1D}-like receptors mediate a number of peripheral effects of serotonin, including endothelium-dependent coronary artery relaxation (10) and prejunctional inhibition of sympathetic norepinephrine release (11). 5-HT_{1D} receptors are known to be G protein linked, coupling, at least in substantia nigra, to inhibition of adenylyl cyclase (12).

Libert *et al.* (13) isolated several novel candidate G protein-linked receptor cDNAs from a canine thyroid library, including one, called RDC4, that was observed to have a sequence most closely related to that of the 5-HT_{1A} receptor (13). We also noted that the RDC4 sequence predicted a polypeptide with a particularly short carboxyl-terminal putative intracellular tail, a feature common to receptors linked to inhibition of adenylyl cyclase via G_i. These features suggested to us that the RDC4 sequence might encode a 5-HT_{1D} receptor. We now describe the cloning of a human gene encoding the homolog of the RDC4 cDNA and show that it encodes a receptor of the 5-HT_{1D} subtype. This receptor interacts with a PTX-sensitive G protein to produce a high agonist affinity state of the receptor and inhibits forskolin-stimulated accumulation of cAMP in a stably transfected mammalian cell line. Some pharmacological features of the cloned receptor suggest that there may be several 5-HT_{1D}-like receptor subtypes.

Materials and Methods

Cloning and sequence determination. The coding region of the canine RDC4 sequence (13) was amplified, using PCR, from dog genomic DNA. The RDC4-based primers corresponded to bases −4 to 21 (5′-ATCGATCTGCAGGATCCTAGAATGTCCCCGCCAAACCAGTCA-3′) and the reverse complement of bases 1119 to 1141 (5′-ATCGATCTGCAGGATCCATCAGACTAGGAGGCTTTCCGG-3′)

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; G protein, guanine nucleotide-binding protein; PCR, polymerase chain reaction; PTX, pertussis toxin; mCPP, 1-(*m*-chlorophenyl)piperazine dihydrochloride; TFMPP, 1-(*m*-trifluoromethylphenyl)piperazine hydrochloride; DPAT, (±)-8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin hydrobromide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SSC, standard saline citrate.

of the published RDC4 sequence, incorporating restriction sites to facilitate subcloning. The identity of the PCR product was confirmed by restriction analysis. The PCR product was random prime labeled using [α-^{32}P]dCTP and was used to screen a human genomic library in Lambda FIX II (Stratagene), with hybridization (6× SSC at 55°; 1× SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7) and washing (2× SSC, 58°) at moderate stringency. Both strands of the reported sequence were determined along their full lengths by dideoxynucleotide termination, using Sequenase (United States Biochemicals) and the usual nucleotides as well as either dITP or 7-deaza-dGTP. Double-stranded plasmid templates and [α-^{35}S]-dATP were used. Amino acid sequence comparison was performed as described in Ref. 14, using a gap penalty of 3 and a length weight of 0.1. Other standard nucleic acid and bacteriological manipulations were as described in Ref. 15.

Expression of the cloned gene in COS-7 and CHO-K1 cells. A 2.0-kb *Pvu*II-*Bgl*II fragment containing the coding region of MA6A was ligated between the *Sma*I and *Bam*HI sites of the eukaryotic expression vector pcD-PS (16). This construction, pcD-MA6A, was used to transfect COS-7 cells, using the DEAE-dextran method (17), or was co-transfected with pSV2Neo in CHO-K1 cells, using calcium phosphate precipitation (15). Transfected COS-7 cells were harvested for binding assays 48–72 hr later. Stable CHO-K1 transfects were selected in the presence of G418 (Geneticin; GIBCO) at 400 μg of base/ml. COS-7 cells were grown in Dulbecco's modified Eagle's medium and CHO-K1 and derivatives in Ham's F-12 medium, both supplemented with 10% dialyzed fetal calf serum (to avoid the high levels of potentially confounding serotonin found in nondialyzed serum), 100 μg/ml penicillin G, and 100 units/ml streptomycin, at 37° in a 5% CO_2 atmosphere.

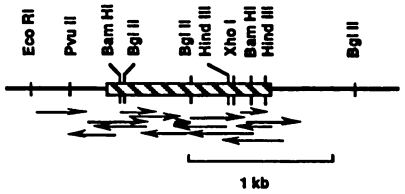
[^{3}H]5-HT binding. Cells were scraped into Dulbecco's phosphate-buffered saline containing 5 mM EDTA, centrifuged at 6000 × *g* for 25 min, and resuspended, by using a Polytron, in 100 mM Tris·HCl, 20 mM $MgSO_4$, 1 mM EDTA (pH 7.7 at 25°). [^{3}H]5-HT (24–27 Ci/mmol; New England Nuclear) filtration binding assays were performed in triplicate, as described in Ref. 18, using 60–400 μg of crude cellular membrane protein/tube. Pargyline (10 μM) had no effect on [^{3}H]5-HT binding to transfected cell membranes and so was not routinely used. Data were analyzed using the nonlinear regression analysis program LIGAND (19), with two-site models accepted only if preferred at $p < 0.02$, using a partial *F* test. Nonspecific binding was determined in the presence of 500 nM metergoline for saturation experiments or by curve-fitting for competition data. Specific binding accounted for 50–85% of total binding at the radioligand K_d. Protein measurement was by the method of Bradford (20), using bovine γ-globulin as standard. Compounds were obtained as described (21) and as gifts, as follows: ICS 205 930 [(3-α-tropanyl)-1*H*-indole-3-carboxylic acid ester] (Sandoz); methiothepin (Hoffman-LaRoche); metergoline (Farmitalia); and 5-carboxamidotryptamine and sumatriptan (GR 43175; 3-[2-dimethylamino]ethyl-*N*-methyl-1*H*-indole-5 methane sulfonamide) (Glaxo). 5-HT, 5-methoxytryptamine, yohimbine, and mCPP were purchased from Sigma; TFMPP from Aldrich; CGS-12066B [7-trifluoromethyl-4-(4-methyl-1-piperazinyl)-pyrrolo[1,2-*a*]quinoxaline] from Research Biochemicals; and PTX from List Biochemicals.

Cyclic AMP accumulation. CHO-K1 cells expressing the 5-HT_{1D} receptor were grown to confluence in 35-mm culture wells (approximately 1.5 × 10^6 cells) and assayed for cAMP accumulation, as described (22), using 100 μM forskolin (Calbiochem) and a 7-min accumulation time, in the presence of HEPES (10 mM)-buffered Ham's F-12 medium. Cyclic AMP was then measured in the cell extract by competitive protein binding (23), using commercial bovine cAMP-dependent protein kinase (Sigma) and [^{3}H]cAMP from Amersham.

Results and Discussion

We amplified a fragment from canine genomic DNA containing the entire canine RDC4 coding region (13), using PCR and appropriate end-specific primers, as described in Materials and Methods. The size of this PCR product, 1.2 kilobases, indicated that there were probably no introns in the canine coding sequence. The use of a genomic library for the isolation and functional characterization of the human homolog of RDC4 was thus attractive. A 16-kilobase human genomic clone in Lambda FIX II, denoted MA6A, was isolated by hybridization at moderate stringency to the PCR-generated RDC4 probe. The MA6A sequence contains a 1131-nucleotide open reading frame corresponding to a predicted 377-amino acid residue protein (Fig. 1). The presumed initiation codon lies within a translation initiation consensus sequence (24) 81 base pairs downstream from an in-frame stop codon. The deduced MA6A amino acid sequence has the hallmark seven hydrophobic regions presumed to represent membrane-spanning domains typical of many G protein-linked receptors (Fig. 1). Such receptors have been proposed to have an extracellular amino terminus and intracellular carboxyl terminus. The amino-terminal region of the MA6A-encoded receptor contains three consensus asparagine-linked glycosylation sites, whereas the presumed second extracellular loop contains one. The portion between the presumed fifth and sixth hydrophobic regions, the putative third cytoplasmic loop, is approximately 83 amino acids in length. This loop has two consensus cAMP-dependent protein kinase sites (25) and a number of other serine and threonine residues that might serve as phosphorylation sites, but no strong consensus recognition sites for protein kinase C or for β-adrenergic receptor kinase (26). The carboxyl-terminal tail distal to the seventh hydrophobic domain is among the shortest of the known G protein-linked receptors, with a predicted length of 19 residues. This tail lacks the cysteine residue conserved in many members of this receptor family.

Several features of the MA6A sequence indicate that it encodes an exact human homolog of the canine RDC4 gene product. The predicted amino acid sequences for MA6A and RDC4 are the same length and are 88% identical, or 93% similar with conservative substitutions. The 5′ and 3′ flanking portions of the MA6A gene also show a high degree of similarity with the RDC4 cDNA untranslated regions. The 102-nucleotide length of MA6A 3′ flanking sequence in Fig. 1 is 69% identical to the corresponding portion of the RDC4 sequence. The 169-nucleotide segment of MA6A immediately upstream of the start codon is 79% identical to the corresponding region of RDC4. Upstream of base −169 in MA6A, similarity to RDC4 breaks down, indicating the possible presence of an intron. Among other reported sequences for members of the G protein-linked receptor family, the most similar to the MA6A sequence is that of the 5-HT_{1A} receptor (2, 3), with which MA6A shares 43% deduced amino acid identity (64% similarity). The presumed membrane-spanning regions are particularly well conserved between the MA6A and 5-HT_{1A} sequences, for which there is 55% identity and 75% similarity. Amino acid sequence identity to the other cloned serotonin receptors was found to be less, at 37% (65% similarity) for the *Drosophila* 5HT-dro receptor (6), 33% (56% similarity) for the rat 5-HT_{1C} receptor (4), and 31% (54% similarity) for the rat 5-HT_2 receptor (5). These sequence similarities parallel the pharmacological similarities among these receptors, in that the drug specificity of 5-HT_{1D} receptors is considerably closer to that of the 5-HT_{1A} receptor than to those of 5-HT_{1C} or 5-HT_2 receptors (27). The cloning of a human 5-HT_{1D} receptor has also been described by Zgombick *et al.* (28) in a preliminary report, although the particulars of

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                                                    AGACCTTAACTACCAGCTGGTAGTTGTCTC  -241
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GTTCTGGTGTACGGCTCCCACCAGTGTGAAGCAGAAGGATGACTTTGCTCTGTTGTCAGG   -61
ACAACCTTGAAGGAAGGAGCCAAATGTGTGGAGGTCTGTGGGAAGAGAGAGCCACCTAGC    -1

ATGTCCCCACTGAACCAGTCAGCAGAAGGCCTTCCCCAGGAGGCCTCCAACAGATCCCTG    60
MetSerProLeuAsnGlnSerAlaGluGlyLeuProGlnGluAlaSerAsnArgSerLeu
     ↑                                          ↑
AATGCCACAGAAACCTCAGAGGCTTGGGATCCCAGGACCCTCCAGGCGCTCAAGATCTCC   120
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 ↑
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ACCACCATCTTACTCACCAGGAAGCTCCACACCCCTGCCAACTACCTGATTGGCTCCCTG   240
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GCCACCACCGACCTCTTGGTTTCCATCTTGGTAATGCCCATCAGCATCGCCTATACCATC   300
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TGCTGCACAGCCTCCATCCTGCATCTCTGTGTCATTGCTCTGGACAGGTACTGGGCAATC   420
CysCysThrAlaSerIleLeuHisLeuCysValIleAlaLeuAspArgTyrTrpAlaIle

ACAGATGCCCTGGAATACAGTAAACGCAGGACGGCTGGCCACGCGGCCACCATGATCGCC   480
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GCCCAGGAGGAGATGTCGGACTGTCTGGTGAACACCTCTCAGATCTCCTACACCATCTAC   600
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                    ↑
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CTTGCTGACAGTGCCCTGGAACGCAAGAGGATTTCTGCTGCTCGAGAAAGGAAAGCCACT   900
LeuAlaAspSerAlaLeuGluArgLysArgIleSerAlaAlaArgGluArgLysAlaThr

AAAATCCTGGGCATCATTCTGGGGGCCTTTATCATCTGCTGGCTGCCCTTCTTCGTGGTG   960
LysIleLeuGlyIleIleLeuGlyAlaPheIleIleCysTrpLeuProPhePheValVal

TCTCTGGTCCTCCCCATCTGCCGGGACTCCTGCTGGATCCACCCGGCGCTCTTTGACTTC  1020
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GAAGAGTTTCGGCAAGCTTTTCAGAAAATTGTCCCTTTCCGGAAGGCCTCCTAGTCTTAT  1140
GluGluPheArgGlnAlaPheGlnLysIleValProPheArgLysAlaSer *

TCGGTGATGACTCTTGTTATCTTTTGTGTCCTGTAACCTCATCGGGATTGTCTTTTTTTT  1200
TTTTAATTATTTTCTGAGACTTGGATTAATTCATGG
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Fig. 1. Restriction map and nucleic acid sequence of clone MA6A. *Top*, the map depicts a portion of clone MA6A, with the 5′ end of the sense strand to the *left* and the coding region *cross-hatched*. The sequencing strategy is denoted by *arrows*. *Bottom*, hydrophobic putative membrane-spanning regions, (*underlined*) were determined as described in Ref. 43, using a window size of 11. Putative asparagine-linked glycosylation sites are shown by *arrows*. Consensus cAMP-dependent protein kinase sites are *double underlined*.

the approach used and sequence information have not been presented.

Membranes prepared from COS-7 cells transfected with the MA6A coding region subcloned in the eukaryotic expression vector pcD-PS were found to express high affinity specific [^{3}H] 5-HT binding sites, whereas in membranes from wild-type COS-7 cells or cells transfected with pcD-PS no specific binding could be detected. In pcD-MA6A-transfected COS-7 cells, non-linear regression analysis (19) of [^{3}H]5-HT saturation isotherms was consistent with the presence of a single class of specific binding sites, with an affinity of 4.8 nM (mean pK_d = 8.32, SD = 0.37, three experiments). B_{max} values varied among different transfections from approximately 60 to 300 fmol/mg of protein. Transfection of COS-7 cells by the canine RDC4 coding region in pcD-PS yielded comparable high affinity specific [^{3}H]5-HT binding, although this was not further characterized.

Competition studies were performed to establish the pharmacological characteristics of the MA6A gene product. Competition for specific [^{3}H]5-HT binding to transfected COS cell membranes by all drugs examined yielded data that were adequately fit assuming a single class of binding sites, using non-linear regression analysis (Table 1). The rank order of potency at the MA6A-encoded receptor (5-carboxamidotryptamine > 5-methoxytryptamine ≈ 5-HT > RU24969 > yohimbine > TFMPP > DPAT > mesulergine, spiperone, ICS 205 930) clearly matches the current definition of a 5-HT$_{1D}$ receptor and is unlike that for any other known subtype (1). Comparison of pK_i values obtained for MA6A binding with those obtained in radioligand binding studies of human brain membrane 5-HT$_{1D}$-type binding sites (18, 27, 29) yields a correlation coefficient of r = 0.96 (employing values for all 12 drugs studied for receptors from both sources). Although the MA6A-encoded receptor, with its relatively low cyanopindolol affinity, does not match the profile of a 5-HT$_{1B}$ receptor (30), it does show high affinity for both CGS 12066B, a compound known to be relatively selective for the 5-HT$_{1B}$-type receptor (31), and dihydroergotamine, a very high 5-HT$_{1B}$ affinity (but quite nonselective) ergopeptine (21). These findings re-emphasize the probable close kinship of the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (32). The 5-HT$_{1B}$ receptor, which has so far been found only in tissue from rats and mice (33) and in an opossum cell line (34), may more properly be thought of as a species variant of the 5-HT$_{1D}$ receptor (32), rather than as a distinct receptor subtype. Cloning of the 5-HT$_{1B}$ receptor will allow comparison of these possible homologs.

We examined whether the cloned 5-HT$_{1D}$ receptor displayed a G$_i$ linkage, as shown by at least some brain 5-HT$_{1D}$ receptors (12). cAMP accumulation is difficult to examine using a transient expression system, because manipulations such as forskolin addition will stimulate cAMP accumulation in all cells, whereas the receptor under study will be expressed in only a small fraction (17). We, therefore, established a clone of CHO-K1 cells permanently expressing the cloned 5-HT$_{1D}$ receptor. Cells were co-transfected with pcD-MA6A and pSV2Neo; positive transfectants were selected in the presence of G418. Of the four clones of G418-resistant transfected cells studied, only one showed substantial levels of [^{3}H]5-HT specific binding, whereas the others and untransfected CHO-K1 cells had no detectable specific [^{3}H]5-HT binding. The cloned 5-HT$_{1D}$ receptor expressed in CHO-K1 cells had similar pharmacological properties as the same clone expressed in COS-7 cells, as shown by the affinities of several key drugs (Table 1).

G protein-linked receptors are commonly found to exist in interconvertible high and low agonist affinity states (35). The high agonist affinity state typically is sensitive to GTP and, in the case of G$_i$-linked receptors, to PTX. [^{3}H]5-HT saturation isotherms using membranes prepared from the CHO-K1 cell

TABLE 1

Drug affinities for [^{3}H]5-HT-labeled sites on membranes of pcD-MA6A-transfected COS-7 and CHO-K1 cells

Drug competition for [^{3}H]5-HT (2–4 nM) binding to cell membranes was performed as described in Materials and Methods. pK_i values are ± standard deviation for n = 3 and ± range/2 for n = 2, where n is the number of independent determinations given in parentheses. All curves were best fit, using nonlinear regression analysis, to a single class of binding sites.

Drug	COS-7 cells		CHO-K1 cells	
	pK_i	K_i	pK_i	K_i
		nM		nM
Dihydroergotamine	9.87 ± 0.01 (3)	0.13		
Metergoline	9.22 ± 0.09 (3)	0.61		
5-Carboxyamidotryptamine	9.01 ± 0.19 (3)	0.99		
5-Methoxytryptamine	8.66 ± 0.14 (3)	2.2		
CGS 12066B	8.57 ± 0.40 (2)	2.7		
Methiothepin	8.24 ± 0.03 (2)	5.8		
5-HT	8.05 ± 0.39 (3)	8.9	8.68 ± 0.05 (2)	2.1
RU 24969	7.66 ± 0.11 (3)	22	7.78 ± 0.16 (2)	18
Yohimbine	7.23 ± 0.18 (3)	59	7.32 ± 0.15 (3)	51
(±)-Cyanopindolol	7.07 ± 0.01 (2)	85		
TFMPP	7.07 ± 0.03 (2)	85		
mCPP	6.67 ± 0.08 (2)	210		
DPAT	6.52 ± 0.07 (2)	300		
Mesulergine	5.77 ± 0.14 (2)	1,700		
Spiperone	5.51 ± 0.08 (2)	3,100		
ICS 205 930	>5 (2)	>10,000		
Sumatriptan			8.43 ± 0.04 (3)	3.7

clone expressing 5-HT$_{1D}$ receptors were best fit assuming the presence of one population of binding sites (Fig. 2). Addition of 1 mM GTP during agonist binding or preincubation of cells with PTX (200 ng/ml, 18 hr) reduced the affinities approximately 3- and 7-fold, respectively. Isotherms performed under these latter two conditions were also best fit by assuming a single class of sites. These data suggest that the cloned 5-HT$_{1D}$ receptor is able to couple to a PTX-sensitive G protein.

In the CHO-K1 cells expressing 5-HT$_{1D}$ receptors, serotonin potently inhibited forskolin-stimulated cAMP accumulation

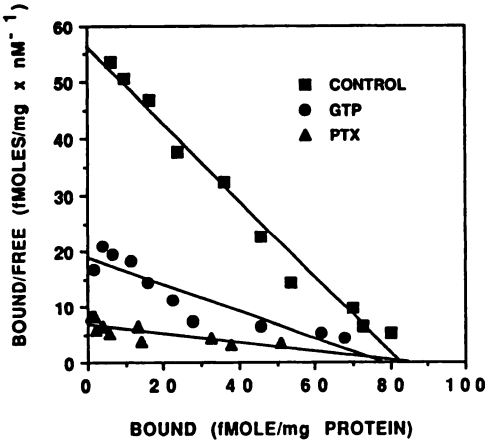

Fig. 2. Effects of PTX and GTP on [^{3}H]5-HT binding to the cloned 5-HT$_{1D}$ receptor stably expressed in CHO-K1 cells. Membranes were prepared from a clone of CHO-K1 cells stably transfected with pcD-MA6A for [^{3}H]5-HT (0.1–15 nM) binding. The data are displayed as a Rosenthal (pseudo-Scatchard) plot. The K_d of the 5-HT$_{1D}$ receptor for [^{3}H]5-HT was lowered from 1.5 nM to 3.9 nM by inclusion of 1 mM neutralized GTP in the binding reaction and to 11 nM by pretreatment of the cells with PTX (200 ng/ml for 18 hr). The B_{max} was not significantly changed by either treatment. The experiment was performed twice with similar results. The data were best fit by assuming a single class of sites in each case, although there was a trend toward curvilinearity consistent with more than one site in the case of GTP. This may indicate an incomplete conversion of high to low agonist affinity state of the receptor under these conditions.

(Fig. 3A). The 5-HT$_{1D}$-active agonists RU 24969 and sumatriptan (GR43175) were also effective in inhibiting forskolin-stimulated cAMP accumulation (Fig. 3B). The putative 5-HT$_{1D}$ competitive antagonist methiothepin had no significant effect on forskolin-stimulated cAMP accumulation but abolished the inhibition produced by 5-HT. Pretreatment of the cells with PTX abolished the serotonin-mediated effect (Fig. 3C). These results strongly indicated that the MA6A-encoded 5-HT$_{1D}$ receptor couples to a PTX-sensitive G$_i$ protein to mediate inhibition of adenylyl cyclase. The structure of the 5-HT$_{1D}$ receptor is in keeping with previously described G$_i$-linked receptors, in that a consistent feature is a very short (14–21-residue) carboxyl-terminal intracellular tail. It is possible that the cloned 5-HT$_{1D}$ could also couple less efficiently to other effectors, as shown for several G$_i$-linked receptors expressed in CHO (36–39) and other cell lines (40, 41).

Despite close general agreement between drug affinities for the MA6A-encoded receptor and published values for 5-HT$_{1D}$ affinities, several discrepancies were noted. K_d values for metergoline and methiothepin were 3–30 times lower than corresponding values obtained using human brain tissue (27, 29). Although no value for the affinity of dihydroergotamine at human brain 5-HT$_{1D}$ binding sites is available, the K_d for the 5-HT$_{1D}$ binding site in calf brain (27) is more than 2 orders of magnitude lower than that for the cloned human receptor. The K_d of the cloned receptor was nearly 2 orders of magnitude lower for sumatriptan and more than 3 orders of magnitude lower for CGS12066 B than the EC$_{50}$ of these drugs in the inhibition of serotonin release via 5-HT$_{1D}$ receptors in animal cerebral cortex slices (8, 42). In part, these discrepancies may reflect species differences, differences inherent in comparisons of agonist binding and effector activation, and differences in assay conditions employed. The magnitude of these discrepancies, however, suggests the existence of more than one serotonin receptor fitting the current definition of 5-HT$_{1D}$ subtype. The identification here of one 5-HT$_{1D}$ receptor sequence may

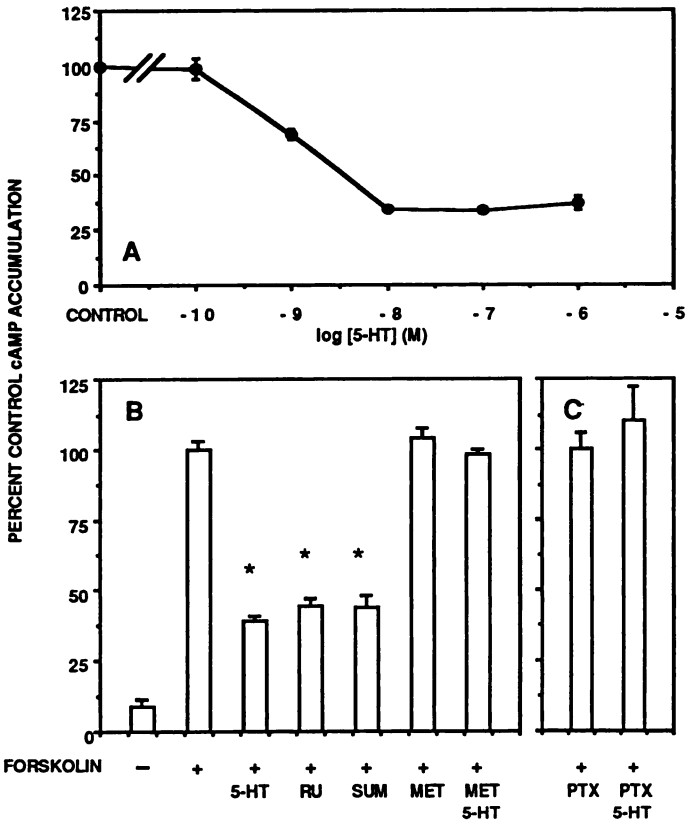

Fig. 3. Inhibition of forskolin-stimulated cyclic AMP accumulation by the cloned 5-HT$_{1D}$ receptor. Forskolin and various drugs were added to adherent CHO-K1 cells stably expressing the cloned 5-HT$_{1D}$ receptor, and cAMP was measured as described in Materials and Methods. Each *point* represents the mean ± standard deviation of results from three wells, each assayed in duplicate. A, Dose response for 5-HT. Basal and forskolin-stimulated cAMP values were 62 ± 6 and 776 ± 11 pmol/dish, respectively. B, Pharmacological specificity. The 5-HT$_{1D}$ agonists RU 24969 (*RU*) and sumatriptan (*SUM*), like 5-HT, inhibited the stimulation of cAMP accumulation elicited by 100 μM forskolin. 5-HT, RU 24969, and sumatriptan were added at a final concentration of 50 nM, whereas methiothepin (*MET*) was used at 1000 nM. In this experiment, the forskolin-stimulated cAMP value was 969 ± 32 pmol/well. *, Values significantly different from the forskolin-only value, $p < 0.01$, Bonferroni modification of Student's *t* test. C, Effect of PTX. Cells were pretreated with PTX (200 ng/ml, 18 hr) before determination of cAMP accumulation. The forskolin-stimulated cAMP value was 599 ± 36 pmol/well. PTX treatment in the absence of serotonergic agonists consistently reduced forskolin-stimulated cAMP accumulation values by 30–40%, compared with forskolin stimulation of untreated cells.

assist in the isolation and characterization of other such closely related receptor genes.

Acknowledgments

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Send reprint requests to: Mark W. Hamblin, GRECC 182B, Seattle VAMC, 1660 South Columbian Way, Seattle, WA 98108.