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ACCELERATED COMMUNICATION

Mouse 5-Hydroxytryptamine$_{5A}$ and 5-Hydroxytryptamine$_{5B}$ Receptors Define a New Family of Serotonin Receptors: Cloning, Functional Expression, and Chromosomal Localization

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SUMMARY

Serotonin [5-hydroxytryptamine (5-HT)] is a neuromodulator that mediates a wide range of physiological functions by activating multiple receptors. Using a strategy based on amino acid sequence homology between 5-HT receptors that interact with guanine nucleotide-binding proteins, we have isolated from a mouse brain library a cDNA encoding a new serotonin receptor. Amino acid sequence comparisons revealed that this receptor was a close relative of the previously identified 5-HT$_5$ receptor but was distant from all other 5-HT receptor subtypes; we therefore named it 5-HT$_{5B}$. When expressed in COS-7 cells, the 5-HT$_{5B}$ receptor displayed a high affinity for the serotonergic radioligand ^{125}I-lysergic acid diethylamide. Its pharmacological profile was distinct from that of all classic 5-HT receptor subtypes. However, the high affinity of the 5-HT$_{5B}$ receptor for 5-carboxamidotryptamine and its low affinity for sumatriptan indicated that it might correspond to recently described 5-HT$_{1D}$-like binding sites that were labeled with [^{3}H]5-carboxamidotryptamine and insensitive to sumatriptan. *In situ* hybridization experiments revealed that the 5-HT$_{5B}$ mRNA was expressed predominantly in the habenula and in the CA1 field of the hippocampus. We also determined the chromosomal localization of the 5-HT$_{5A}$ and 5-HT$_{5B}$ genes and of their human counterparts. The 5-HT$_{5A}$ gene colocalized with the mouse mutation reeler and the human mutation holoprosencephaly type 3, which both result in abnormal brain development, raising the possibility that the 5-HT$_{5A}$ receptor plays a role in brain development.

Serotonin is a neuromodulator that elicits and modulates a wide range of behaviors such as sleep, appetite, locomotion, sexual activity, and vascular contraction (1). Both pharmacological studies and molecular cloning of 5-HT receptors have revealed a multiplicity of receptor subtypes. The receptors that have been cloned so far belong either to the ligand-gated ion channel family (5-HT$_3$ receptors) or to the large family of receptors that interact with G proteins and share a putative seven-transmembrane domain structure (2). Amino acid sequence comparisons have revealed that the G protein-coupled 5-HT receptors can be subdivided into two distinct groups, i.e., the 5-HT$_1$ group, which contains the mammalian 5-HT$_{1A}$ (3), 5-HT$_{1B}$ (4), 5-HT$_{1D}$ (5), 5-HT$_{1E\alpha}$ (6), and 5-HT$_{1E\beta}$ (7) subtypes as well as three *Drosophila* 5-HT receptors (8), and the 5-HT$_2$ group, which contains the 5-HT$_2$ (9), 5-HT$_{1C}$ (10), and 5-HT$_{2F}$ (11) subtypes. These receptors differ in their affinity for serotonin, their intracellular signaling properties, their pattern of expression, and their subcellular localization (see Ref. 12 for a review).

Pharmacological studies have revealed the existence of additional receptor subtypes such as the 5-HT$_4$ receptor, as well as a number of "5-HT$_{1D}$-like" receptors (see Ref. 13 for a review). To isolate some of these subtypes, we used a strategy based on nucleotide sequence homology between transmembrane domains III and VI of 5-HT receptors. A mouse brain cDNA library was screened and one of the resulting clones was shown to encode a functional 5-HT receptor. Sequence comparisons revealed that this receptor is a new member of the G protein-coupled receptor family that does not belong to the 5-HT$_1$ or 5-HT$_2$ families and that is most homologous (69% amino acid identity) to the recently isolated 5-HT$_5$ receptor (14). We therefore named this receptor 5-HT$_{5B}$ and we renamed the 5-HT$_5$ receptor 5-HT$_{5A}$.

When expressed in COS-7 cells, the 5-HT$_{5B}$ receptor displayed a high affinity for ^{125}I-LSD. Its pharmacological profile was similar to that of the 5-HT$_{5A}$ receptor but did not corre-

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 5-CT, 5-carboxamidotryptamine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; G protein, guanine nucleotide-binding protein; LSD, lysergic acid diethylamide; RU24969, 5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole; kb, kilobase(s).

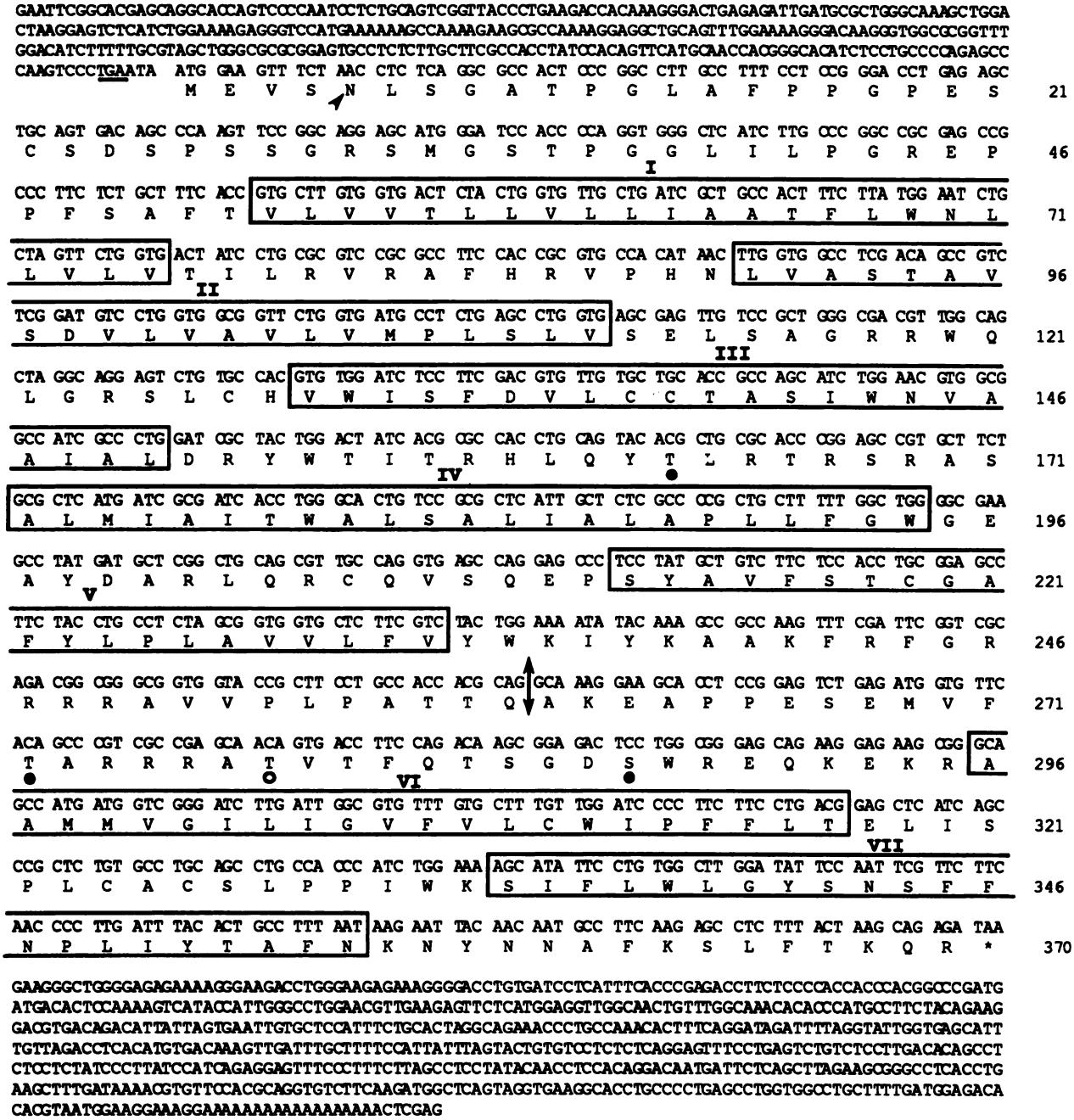

Fig. 1. Nucleotide sequence of the 5-HT_{5B} cDNA. The 2055-base pair *Eco*RI-*Xho*I cDNA fragment was sequenced on both strands. The seven putative transmembrane domains are *boxed and numbered* (*I* to *VII*). *Double-headed arrow*, intron-exon boundary. *Arrowhead*, site of potential *N*-linked glycosylation. *Closed and open circles*, consensus sites for phosphorylation by protein kinases C and A, respectively. An in-frame stop codon upstream of the ATG is *underlined*. *, Terminal stop codon.

spond to the profile of any other serotonin receptor subtype. *In situ* hybridization experiments performed on adult mouse brain sections revealed that the 5-HT_{5B} mRNA was found predominantly in the hippocampus and in the habenula.

Our results therefore reveal the existence of a new family of serotonin receptors, the 5-HT_5 family, that differs from the 5-HT_1, 5-HT_2, and 5-HT_3 families in terms of amino acid sequence and pharmacological profile. Finally, we determined the chromosomal localization of the mouse 5-HT_{5A} and 5-HT_{5B} genes and of their human homologues. The localization of the 5-HT_{5A} gene suggests a possible role for this receptor in brain development.

Materials and Methods

Isolation and sequence of the 5-HT_{5B} cDNA and genomic DNA. A nested polymerase chain reaction experiment was performed with the following oligonucleotides: 1) AGAACTAGTGGATCCAA(A/G)AA(A/G/C/T)GG(A/G/C/T)A(A/G)CCA(A/G)CA, 2) CTTGATATCGAATTCGA(T/C)(A/G)T(A/G/C/T)CT(A/G/C/T)TG(C/T)TG(C/T)AC, and 3) GGTATCGATAAGCTTAT(C/T/A)GC(C/T)CT(A/G/C/T)GA(C/T)(C/A)G(A/G/C/T)TA. Five micrograms of adult mouse brain RNA were reverse transcribed in the presence of 500 ng of oligonucleotide 1 and 200 units of Moloney Murine Leukemia Virus reverse transcriptase (BRL). Half of that reaction was then amplified for 30 cycles in the presence of *Thermus aquaticus* polymerase

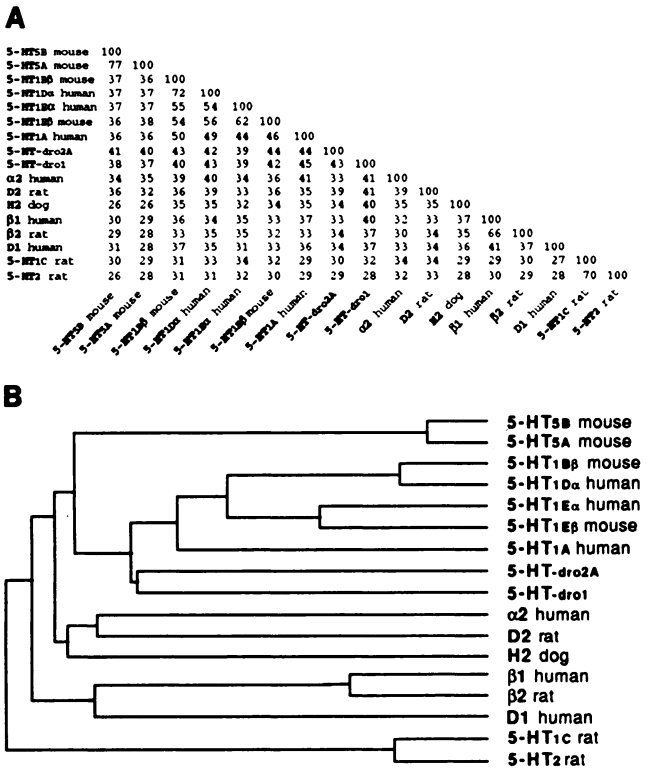

Fig. 2. A, Percentages of amino acid homologies between the 5-HT$_{5B}$ receptor and other members of the G protein-coupled receptor family. These percentages of homology were calculated over the sequences that are conserved in this gene family, i.e., the transmembrane domains and short connecting loops (12). B, Dendrogram. The sequences of the mouse 5-HT$_{5B}$, mouse 5-HT$_{5A}$ (14), mouse 5-HT$_{1B\beta}$ (4), human 5-HT$_{1D\alpha}$ (5), human 5-HT$_{1E\alpha}$ (6), mouse 5-HT$_{1E\beta}$ (7), human 5-HT$_{1A}$ (3), *Drosophila* 5-HT$_{dro1}$ and 5-HT$_{dro2A}$ (8), human α_{2A}-adrenergic (28), rat D2 dopaminergic (29), dog H2 histaminergic (30), human β_1-adrenergic (31), rat β_2-adrenergic (32), human D1 dopaminergic (33), rat 5-HT$_{1C}$ (10), and rat 5-HT$_2$ (9) receptors were compared and clustered with the program Clustal (34). The lengths of the *horizontal lines* are inversely proportional to the percentages of homology (A).

(5 units; Cetus) and oligonucleotides 1 and 2 (1 μg of each). One twentieth of that reaction was amplified for 30 more cycles with oligonucleotides 1 and 3. The resulting products were digested with *Bam*HI and *Hind*III, inserted in the Bluescript plasmid, and sequenced. One of the fragments that exhibited homology with 5-HT receptors was labeled by random priming and used to screen a mouse brain cDNA library constructed in the Uni-Zap phage (Stratagene). Positive phages were isolated and the cDNA inserts were recovered in the Bluescript plasmid and sequenced on both strands by the dideoxynucleotide technique, using successive synthetic oligonucleotides.

To isolate the 5-HT$_{5A}$ and 5-HT$_{5B}$ genomic clones we screened a genomic library prepared from a partial *Sau*3A digest of genomic DNA from mouse embryonic stem cells, inserted in the EMBL3 λ phage. The probes used were the 5-HT$_{5A}$ (14) and 5-HT$_{5B}$ cDNA fragments. DNA fragments that hybridized with these probes were subcloned in the Bluescript plasmid and partially sequenced.

Chromosomal localization. The 5-HT$_{5A}$ and 5-HT$_{5B}$ probes corresponded to the 4.0-kb 5-HT$_{5A}$ cDNA (14) and 2.1-kb 5-HT$_{5B}$ cDNA fragments, respectively; they were tritium labeled by nick translation and hybridized to metaphase spreads, as described previously (15).

Expression of the 5-HT$_{5B}$ receptor in COS-7 cells. The *Eco*RI-*Xho*I cDNA fragment (Fig. 1) was inserted between the *Eco*RI and *Xho*I sites of expression vector p513, which is a derivative of pSG5 (16) containing a multiple cloning site. The resulting recombinant was introduced into COS-7 cells by calcium phosphate-mediated transfection (20 μg/10-cm dish), and the cells were harvested 48 hr after transfection.

Radioligand binding assay. Membranes were prepared as described (17). ^{125}I-LSD saturation and competition binding experiments were performed with 10–20 μg of protein/sample in 50 mM Tris·HCl, pH 7.4, in a final volume of 250 μl, at 37° for 10 min. Reactions were terminated by filtration under vacuum over Whatman GF/C glass fiber filters, which were rinsed four times with 4 ml of 50 mM Tris·HCl, pH 7.4. Nonspecific binding was defined with 10 μM 5-HT. Radioactivity was determined in a γ counter.

***In situ* hybridization.** *In situ* hybridizations were performed on cryostat sections of adult mouse brains (about 8 weeks of age) as described (18). The 5-HT$_{5B}$ sense probe used was a single-stranded RNA probe produced in the presence of ^{35}S-CTP and T3 polymerase, using as a template the full length cDNA plasmid linearized with *Xho*I (Fig. 1). The antisense probe was produced in the presence of T7 polymerase using a template linearized with *Eco*RI. The 5-HT$_{5A}$ probe was prepared in the same way, from a template containing the full length 5-HT$_{5A}$ cDNA (14).

Results

Isolation of a mouse cDNA clone encoding a new member of the G protein-coupled receptor family, the 5-HT$_{5B}$ receptor. Sequence comparisons of serotonin receptors have revealed a striking amino acid sequence conservation, particularly in certain putative transmembrane domains such as domains III and VI (12). We therefore decided to use degenerate oligonucleotides corresponding to these two regions to perform a series of polymerase chain reaction experiments on mouse brain RNA. The resulting fragments were subcloned and sequenced. One of these fragments was used to screen a mouse brain cDNA library. We obtained a phage recombinant that contained a 2.1-kb cDNA insert. Sequence analysis revealed one long open reading frame (370 amino acids) and a poly(A)$^+$ tail (Fig. 1). The hydropathy analysis of this predicted protein revealed seven hydrophobic domains (numbered I to VII in Fig. 1), a feature shared by all other cloned members of the G protein-coupled receptor family. The amino-terminal end displayed one putative site for *N*-linked glycosylation and the presumed cytoplasmic domains contained consensus sites for phosphorylation by protein kinases C and A (Fig. 1), features that are found in most members of that family.

Amino acid sequence comparisons revealed homologies with G protein-coupled receptors in the putative transmembrane domains and short connecting loops but not in the amino- and carboxyl-terminal ends or the third cytoplasmic loop, which are very variable in sequence and in length within this gene family. Percentages of homology were, therefore, calculated over the conserved regions (Fig. 2A) and used to establish a dendrogram (Fig. 2B). The 5-HT$_{5B}$ receptor was highly homologous to the 5-HT$_{5A}$ receptor (77%), whereas the percentages of homology with other known receptors were low, with the best score being 41% with the *Drosophila* serotonin receptor 5-HT$_{dro2A}$ (8). The dendrogram clearly shows that the 5-HT$_{5B}$ receptor does not belong to a subfamily of serotonin receptors such as, for example, the 5-HT$_{1B}$/5-HT$_{1D}$ family or the 5-HT$_2$/5-HT$_{1C}$ family.

Comparisons of the 5-HT$_{5A}$ and 5-HT$_{5B}$ sequences (Fig. 3A) revealed that the homologies were highest in the transmembrane domains and lower in the amino-terminal end and in the middle of the third cytoplasmic loop, two areas that do not appear to confer specificity in other members of the G protein-coupled receptor family.

Genomic structure and chromosomal localization of the 5-HT$_{5A}$ and 5-HT$_{5B}$ genes. In order to isolate genomic

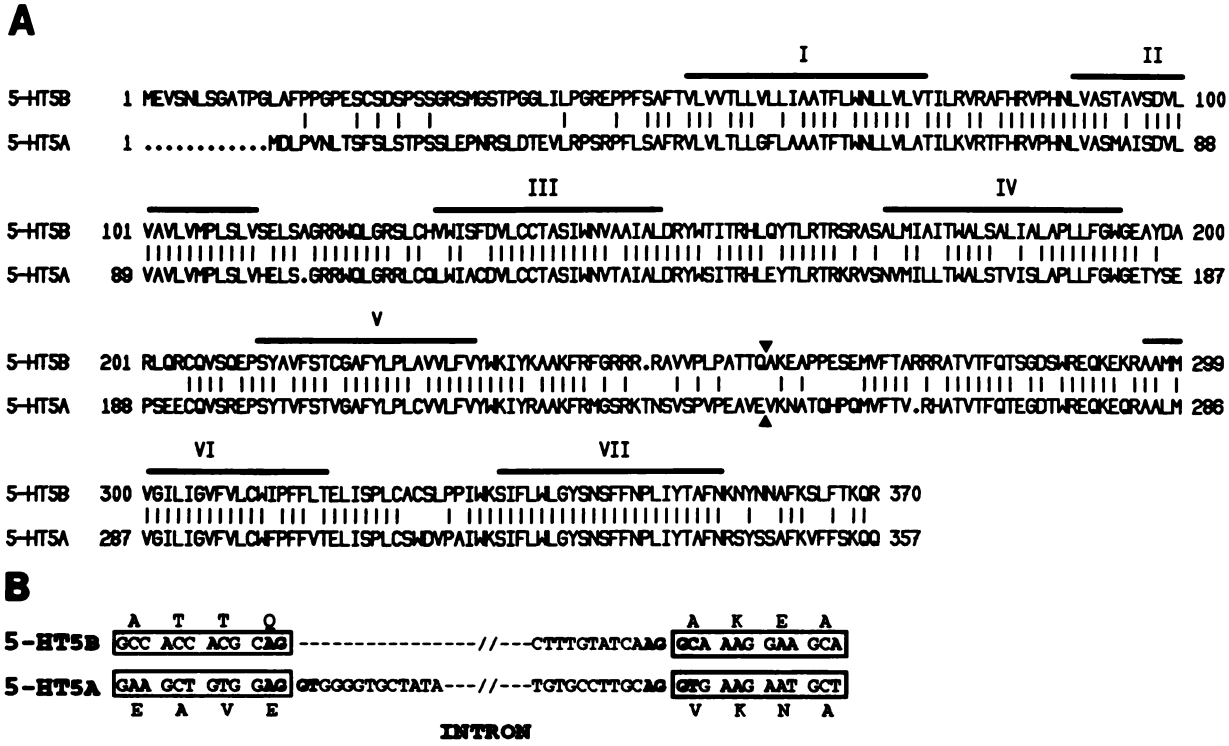

Fig. 3. A, Amino acid sequence comparison between the 5-HT_{5A} and the 5-HT_{5B} receptors. *Vertical bars*, identical amino acids. There is a 69% identity between the 5-HT_{5A} and 5-HT_{5B} receptors. The putative transmembrane domains are indicated (*I-VII*). *Arrowheads between transmembrane domains 5 and 6*, an intron-exon boundary. B, Sequence of the intron-exon boundaries of the 5-HT_{5A} and 5-HT_{5B} receptors.

fragments containing the 5-HT_{5A} and 5-HT_{5B} genes, we screened a mouse genomic library with probes corresponding to the 5-HT_{5A} and 5-HT_{5B} cDNAs. Partial sequence analysis revealed that both the 5-HT_{5A} and 5-HT_{5B} genes contained an intron located in the middle of the third cytoplasmic loop, at exactly the same position in both genes (Fig. 3B). In the case of the 5-HT_{5A} gene, our data indicate that the coding region is interrupted by only one intron, which is about 8 kb long.

We also analyzed the chromosomal localization of the 5-HT_{5A} and 5-HT_{5B} genes in mice and in humans by using the mouse 5-HT_{5A} and 5-HT_{5B} cDNAs as probes. *In situ* hybridization experiments performed on mouse and human chromosomes revealed that in both cases a single locus was labeled (Fig. 4). The 5-HT_{5A} gene was localized on mouse chromosome 5, at position 5B, whereas the putative human counterpart of this gene was found on chromosome 7, at position 7q36. The mouse 5-HT_{5B} gene was localized on chromosome 1 (position 1F), whereas its human homologue was on chromosome 2 (position 2q11–13).

Pharmacological profile of the 5-HT_{5B} receptor. To determine whether the 5-HT_{5B} cDNA clone encoded a functional receptor, we introduced it into a eukaryotic expression vector and transfected COS-7 cells with the resulting recombinant. Membranes of transfected cells were then assayed for their ability to bind a number of serotonergic radioligands. Although we could not detect any binding of [^{3}H]5-HT, ^{125}I-cyanopindolol, [^{3}H]8-OH-DPAT, or [^{3}H]spiperone to these membranes, ^{125}I-LSD displayed a single saturable binding site (K_d = 470 pM and B_{max} = 170 fmol/mg of membrane protein) (Fig. 5). In a control experiment, ^{125}I-LSD did not bind to mock-transfected COS-7 cells. To determine the pharmacological profile of the 5-HT_{5B} receptor, bound ^{125}I-LSD was displaced with various serotonergic drugs (Table 1). These compounds displayed the following rank order of potencies: ergotamine > 5-CT > methysergide > 5-HT > RU24969 = 8-OH-DPAT > yohimbine > bufotenine (Table 1). Ketanserin, sumatriptan, (−)-propranolol, dopamine, and (−)-norepinephrine were inactive.

Competition curves obtained with 5-HT and 5-CT were slightly biphasic, indicating that a small fraction of the ^{125}I-LSD binding sites might have a high affinity for these compounds. However, the fraction of high affinity sites was variable and too small to allow a determination of the high affinity K_i values using the software EBDA/LIGAND (Biosoft).

Expression of the 5-HT_{5B} receptor in the central nervous system. Expression of 5-HT_{5B} transcripts was analyzed by Northern blotting and by *in situ* hybridization experiments. The Northern analysis performed on poly(A)$^+$ RNA samples extracted from various adult mouse tissues (brain, heart, kidney, lung, liver, and intestine) did not reveal any transcript in these organs (data not shown).

Because the 5-HT_{5B} cDNA had been isolated from a mouse brain cDNA library, we performed *in situ* hybridization experiments on mouse brain sections. Expression of the 5-HT_{5B} RNA was detected exclusively in the CA1 field of the hippocampus, the habenula, and the dorsal raphe (Fig. 6A). The specificity of this pattern of expression is demonstrated by the fact that no signal was observed with a "sense probe" (data not shown).

Discussion

Our data indicate that we have isolated a functional serotonin receptor that is expressed predominantly in the central nervous system. The sequence of this receptor reveals that it is a new member of the large family of proteins with seven putative transmembrane domains and that it is most homologous to the

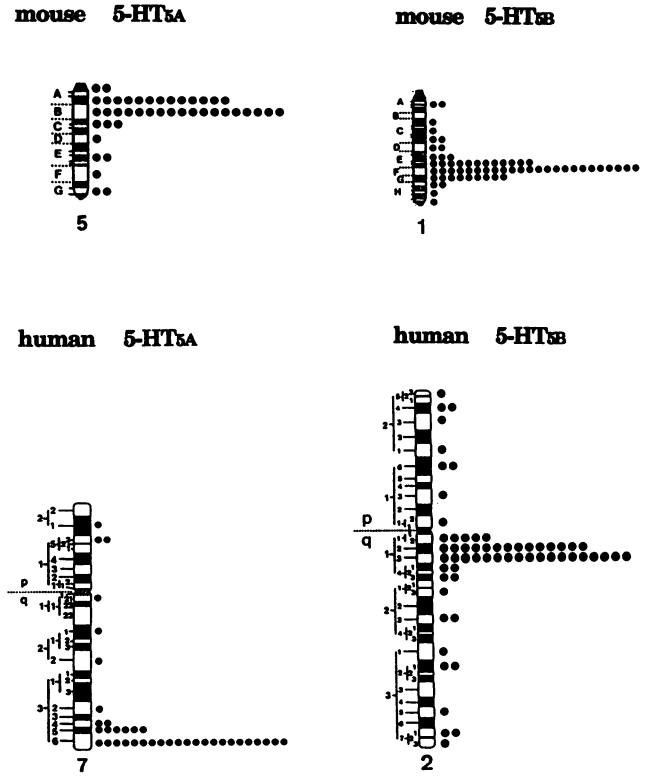

Fig. 4. Chromosomal localization of the 5-HT$_{5A}$ and 5-HT$_{5B}$ receptors. Probes were prepared and hybridized to mouse or human chromosomes as described in Materials and Methods. Diagrams of mouse and human chromosomes are shown, illustrating the distribution of the labeled sites. The 5-HT$_{5A}$ probe hybridized preferentially to the A3-B region of mouse chromosome 5, with a maximum in the B band, and to the q35-q36 region of human chromosome 7, with a maximum in the q36 band. The 5-HT$_{5B}$ probe hybridized to the E4-G region of mouse chromosome 1, with a maximum in the F band, and to the q11-q13 region of human chromosome 2, with a maximum in the q13 band.

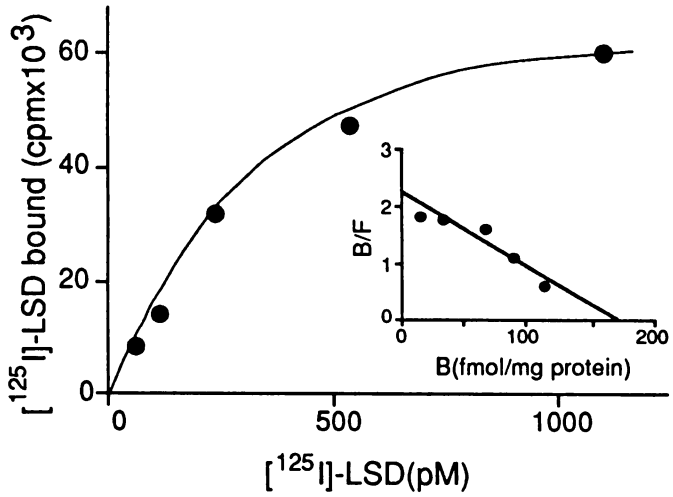

Fig. 5. Saturation isotherm of ^{125}I-LSD binding to membranes of COS-7 cells expressing the 5-HT$_{5B}$ receptor. Membranes were incubated with concentrations of ^{125}I-LSD ranging from 50 pM to 1.25 nM, with or without 10 μM 5-HT. Specific binding is represented. *Inset*, Scatchard analysis of ^{125}I-LSD binding (K_d = 470 pM, B_{max} = 170 fmol of receptor/mg of membrane protein). Data are representative of two independent experiments, with each *point* being measured in triplicate.

TABLE 1

Pharmacological profile in the 5-HT$_{5A}$ and 5-HT$_{5B}$ receptors

Data correspond to competition for ^{125}I-LSD binding to membranes of COS-7 cells transiently expressing the 5-HT$_{5A}$ and 5-HT$_{5B}$ receptors. IC$_{50}$ values required to displace 50% of ^{125}I-LSD were determined experimentally and converted to pK_i values according to the equation $K_i = IC_{50}/(1 + C/K_d)$, where C is the ^{125}I-LSD concentration (150 pM) and K_d is the equilibrium dissociation constant of ^{125}I-LSD. Numbers in parentheses correspond to the number of independent experiments, with each point being measured in triplicate. Individual pK_i values differed by <20%.

	pK_i values	
	5-HT$_{5A}$ (COS-7 cells)	5-HT$_{5B}$ (COS-7 cells)
5-HT	6.6 (7)	6.6 (3)
5-CT	7.8 (3)	7.4 (3)
RU24969	6.5 (2)	6.4 (2)
TFMPP[a]	5.6 (2)	5.4 (2)
8-OH-DPAT	5.9 (2)	6.4 (2)
Sumatriptan	4.8 (2)	5.1 (3)
Bufotenine	6.0 (2)	5.8 (2)
Methysergide	7.2 (5)	6.9 (2)
Ergotamine	8.4 (2)	8.5 (2)
Methiothepin	7.0 (2)	7.8 (3)
Yohimbine	6.0 (2)	6.0 (2)
(−)-Propranolol	4.7 (2)	5.2 (2)
Ketanserin	4.8 (2)	5.8 (2)
Dopamine	4.1 (2)	<5 (2)
(−)-Norepinephrine	2.8 (2)	<5 (2)

[a] TFMPP, 1-(3-trifluoromethylphenyl)piperazine.

recently characterized mouse 5-HT$_5$ receptor (14). We therefore named these two receptors 5-HT$_{5A}$ and 5-HT$_{5B}$. The closest relatives of the 5-HT$_{5A}$ and 5-HT$_{5B}$ receptors are serotonin receptors, but the percentages of homology are small. These two receptors, therefore, define a new subfamily of serotonin receptors that is distinct from other subfamilies such as the 5-HT$_{1B}$/5-HT$_{1D}$ family or the 5-HT$_{1C}$/5-HT$_2$ family. The genomic organization of the 5-HT$_{5A}$ and 5-HT$_{5B}$ genes is different from that of the other 5-HT receptors. Their coding region is interrupted by one intron, whereas no introns were found in the 5-HT$_1$ genes (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, and 5-HT$_{1E}$) and several introns were found in the 5-HT$_2$ genes (5-HT$_{1C}$ and 5-HT$_2$). Despite their resemblance, the 5-HT$_{5A}$ and 5-HT$_{5B}$ genes are not located on the same mouse chromosomes. The 5-HT$_{5A}$ gene is on chromosome 5, whereas the 5-HT$_{5B}$ gene is on chromosome 1. The mouse reeler mutation has been mapped in the same area (5B) as the 5-HT$_{5A}$ gene (19). These mutants exhibit an abnormal brain development, particularly in the cerebellum, cerebral cortex, hippocampus, and olfactory bulb, which are regions where the 5-HT$_{5A}$ receptor is expressed. We are currently analyzing the DNA of reeler mutants to determine whether the 5-HT$_{5A}$ gene is mutated. We have also shown that the mouse 5-HT$_{5A}$ and 5-HT$_{5B}$ genes hybridize to human chromosomes at positions 7q36 and 2q11–13, respectively. This observation suggests the existence of human homologues for both the 5-HT$_{5A}$ and 5-HT$_{5B}$ genes. The human mutation for holoprosencephaly type III (20), which consists of an abnormal development of the brain, is localized in the same region as the 5-HT$_{5A}$ gene, raising the possibility that this gene is mutated in cases of holoprosencephaly.

The pharmacological profile of the 5-HT$_{5B}$ receptor is, as expected, similar to that of the 5-HT$_{5A}$ receptor (Table 1). This profile does not correspond to that of any of the classic 5-HT receptor subtypes, i.e., 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, 5-HT$_{1D}$, 5-HT$_{1E}$, 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$. The 5-HT$_{5B}$ receptor has a high affinity for ergot compounds such as iodinated LSD, ergotamine, and methysergide, which are rather nonspecific 5-

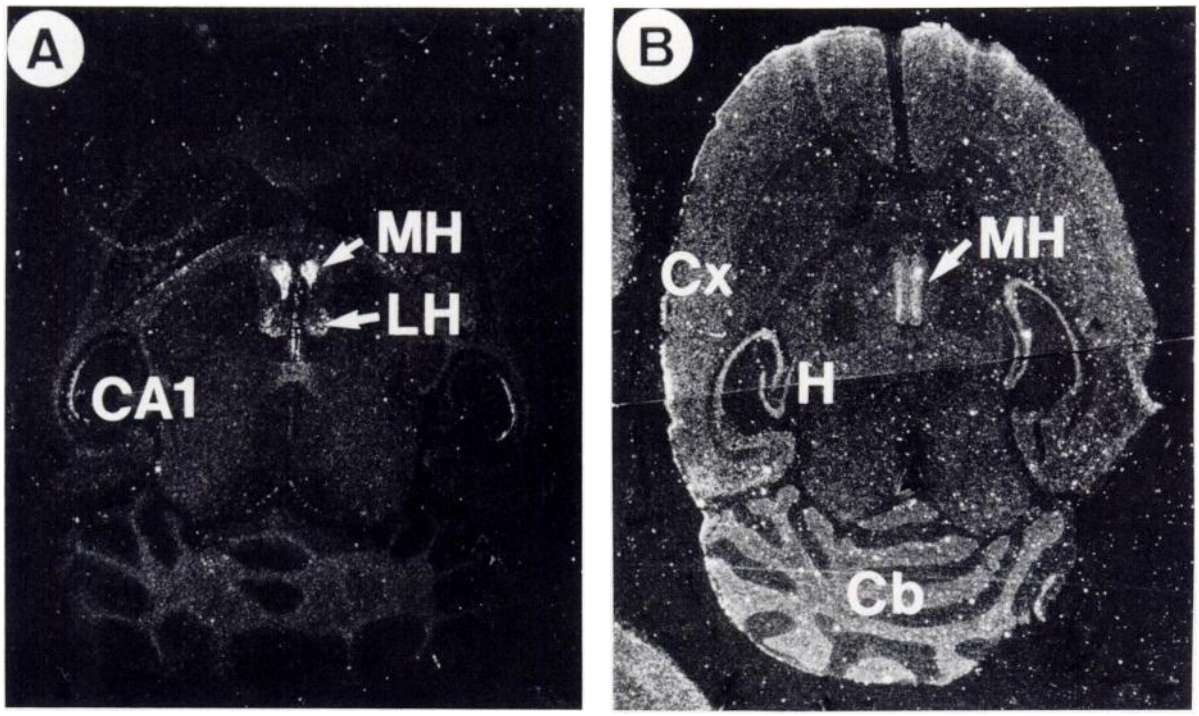

Fig. 6. *In situ* hybridization. A and B, Dark-field microscopy of the emulsion autoradiogram of a horizontal section through an adult mouse brain (8 mm wide), hybridized with either the 5-HT_{5B} probe (A) or the 5-HT_{5A} probe (B). The RNA probe was prepared as described in Materials and Methods. *Cx*, cerebral cortex; *H*, hippocampus; *Cb*, cerebellum; *CA1*, hippocampal area; *MH*, median habenula; *LH*, lateral habenula.

HT ligands. It also has a fairly high affinity for 5-CT, which would classify it in the 5-HT_1 family, but unlike 5-HT_1 receptors it has a low affinity for 5-HT. However, it is noteworthy that 5-HT and 5-CT yielded displacement curves that were slightly biphasic, suggesting the existence of a small fraction of high affinity sites that might correspond to receptors that are coupled to G proteins. We made a similar observation in the case of the 5-HT_{5A} receptor, where we showed that in cell lines expressing low levels of this receptor a fraction of the 5-HT_{5A} sites had a high affinity for 5-HT and 5-CT and were coupled to G proteins (14). The fact that the majority of the 5-HT_{5A} and 5-HT_{5B} receptors might not be coupled to G proteins in COS-7 cells could be explained either by the overexpression of these receptors in the transient COS-7 expression system or by the absence of the appropriate G protein in this system. The first hypothesis seems unlikely because the level of expression of the 5-HT_{5B} receptor was low in COS-7 cells (170 fmol of receptor/mg of membrane proteins). Under such conditions other receptors, such as the 5-HT_{1B} receptor, display a much larger fraction of high affinity sites (21),[1] possibly because they can interact with G proteins in COS-7 cells. Concerning the second hypothesis, it is worth noting that a number of G proteins, such as G_o and G_x, that are found predominantly in neurons are not expressed in COS-7 cells. The 5-HT_5 receptors might couple preferentially to these G proteins. Consistent with this hypothesis is the fact that we could not detect any effect of the 5-HT_{5A} receptor on either cAMP or inositol trisphosphate levels in NIH-3T3 cells stably expressing this receptor (14). These negative results suggest that the 5-HT_{5A} receptor cannot couple efficiently to either G_s, G_i, or G_q proteins. The 5-HT_{5B} receptor, because of its high level of homology with the 5-HT_{5A} receptor, is likely to interact with second messengers in the same way as does the 5-HT_{5A} receptor.

It has recently been shown that [^{3}H]5-CT labels a heterogeneous population of sites in the cerebral cortex and hippocampus of several mammalian species (22, 23). Some of these sites display a high affinity for sumatriptan and most likely correspond to 5-HT_{1D} receptors, whereas others have a low affinity for sumatriptan. These sites that have a high affinity for 5-CT and a low affinity for sumatriptan could possibly correspond to the 5-HT_5 receptors. The pattern of expression of the 5-HT_5 receptors is compatible with this hypothesis, because the 5-HT_{5A} and 5-HT_{5B} mRNAs were found in brain areas that contain 5-CT-sensitive sites such as the cerebral cortex and hippocampus (Fig. 6). In addition, the 5-HT_5 receptors might be responsible for some of the 5-HT_{1D}-like sites, with a low affinity for sumatriptan, RU24969, or 1-(3-trifluoromethylphenyl)piperazine, that have been characterized in brain membranes from various mammalian species including humans (24, 25).

Because we have not been able to detect coupling of the 5-HT_{5A} receptor to either adenylate cyclase or phospholipase C in fibroblasts, it is unlikely that the 5-HT_{5B} receptor interacts with these effector systems. It is therefore possible that both the 5-HT_{5A} and 5-HT_{5B} receptors are coupled to ion channels. Consistent with this idea is the fact that in the hippocampus 5-HT_{1D}-like receptors have been shown to modulate potassium conductances (26). To investigate this possibility we are currently expressing the 5-HT_5 receptors in various neuronal cell lines. Concerning the function of the 5-HT_5 receptors, because of the possible similarity between these receptors and 5-HT_{1D}-like receptors it is conceivable that some of the effects attributed to the 5-HT_{1D} receptors, such as their involvement in feeding, anxiety, and depression (1), might actually be mediated by the 5-HT_5 receptors.

The availability of the genes encoding two members of a new family of serotonin receptors should allow us, via gene-targeting

[1] R. Hen, unpublished observations.

techniques, to generate mutant mice that do not express these receptors and to analyze the consequence of such mutations on physiology and behavior (27).

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Note added in proof. The sequence data from the 5-HT$_5$ receptors will be available (1.4.93) from the EMBL/GenBank squence data bases under the accession numbers Z18278 (5-HT$_{5A}$) and X69867 (5-HT$_{5B}$).

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