Characterization of the Human 5-Hydroxytryptamine_{1B} Receptor*

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We report the cloning of a human gene encoding the 5-hydroxytryptamine{1B} receptor. The receptor has the characteristics of a G-protein-linked receptor and is most homologous to the human 5-HT_{1B} receptor. This human 5-HT receptor gene, most abundantly expressed in striatum, is localized on chromosome 6, at 6q13, and the gene encoding the 5-HT_{1D} receptor is localized on chromosome 1. Radioligand studies indicate that the affinity of [3H]5-HT is 16 ± 2 nM. Drug competition studies indicate that the receptor displays high affinity (i.e. <40 nM) for 5-HT, 5-carboxamidotryptamine, methiothepin, and metergonol.

5-Hydroxytryptamine (5-HT)¹ is a neurotransmitter regulating sensory, motor, and cortical functions (1, 2). Analysis of 5-HT receptor subtypes should elucidate the pathophysiological basis of many diseases such as anxiety, depression, migraine, vasospasm, and epilepsy (3) and will help to develop potent and selective therapeutic agents in neuropsychiatry. Cloning studies have identified four homologous genes, namely 5-HT_{1A} (4, 5), 5-HT_{1C} (6), 5-HT_{3} (7), and recently the 5-HT_{1D} (8) receptors (reviewed in Refs. 9 and 10).

We now describe the cloning of a gene which encodes the human 5-HT_{1B} receptor. We also report the tissue distribution in human, monkey, and rat brain of this receptor and chromosomal localization of both the 5-HT_{1B} and 5-HT_{1D} receptors.

**EXPERIMENTAL PROCEDURES**

PCR—Genomic DNA was amplified by PCR (11) with oligonucleotides (Fig. 1, legend) devised from sequences corresponding to the third and sixth transmembrane regions (TM3 and TM6) of 5-HT receptors. The timing of PCR was 1.5 min at 93 °C, 2 min at 55 °C, and 4 min at 72 °C as described (12). After 30 cycles this DNA was subcloned into the plasmid Bluescript as previously described (13). The ligation mixture was transformed into DH5αF’ bacteria (Bethesda Research Laboratories) and plated out on LB plates containing ampicillin as described (14). Nitrocellulose filters (Millipore) were used to lift bacterial colonies, and the filters were incubated in prehybridization and hybridization solution with nick-translated fragments (14). The filters were then washed in 2 × SSC, 0.1% SDS at room temperature for 20 min, in 0.1 × SSC, 0.1% SDS at 52 °C for 20 min, and exposed to x-ray film overnight at −70 °C with intensifying screens. The inserts were sequenced using Sequenase version 2 (U. S. Biochemical Corp.).

Screening Genomic Library and Southern Blot—A human λEMBL genomic library (Clontech) was screened (15), positive clones were picked, and DNA was prepared from purified plaques (16), cut with Xhol and SacI to excise the inserts, run on nuclease (Genlan Sciences), UV-linked (Stratagene), and hybridized (14).

Expression in Mammalian Cells—Clones isolated from the library screening were subcloned into the expression vector pRK (17, 18) and transformed by the calcium phosphate precipitation method as described previously (18) into 293 human embryonic kidney cells for transient expression. After 48 h, the cells were harvested, homogenized in 20 mM Tris-HCl (pH 7.4), 5 mM EDTA, and sonicated for 10 s. Nuclear debris and intact cells were removed by centrifugation at 1,000 × g for 10 min. The supernatants were centrifuged at 35,000 × g for 30 min, and the resulting pellet was resuspended in binding buffer containing 50 mM Tris-HCl (pH 7.4), 4 mM CaCl₂, 0.1% ascorbic acid, 10 mM pargyline, and 1 mM leupetine.

Preparation of Tissue Suspension—A cell suspension in 50 mM Tris-HCl buffer (about 20 million cells/ml) was thawed from −20 °C and homogenized in 20 volumes of 50 mM Tris-HCl buffer (pH 7.8 at 25 °C) using a Brinkmann Polytron and then centrifuged at 39,000 × g for 10 min at 4 °C. The supernatants were discarded, and the pellets were resuspended in 10 volumes of assay buffer consisting of 50 mM Tris-HCl, 14 mM CaCl₂, 0.1% ascorbic acid, and 10 mM pargyline and immediately used for radioligand binding studies.

Radioligand Binding Assays—Assays consisted of 0.1 m of radioligand (final concentrations: [3H]5-HT, 0.01–150 nM, 0.8 m of tissue suspension, and 0.1 m of assay buffer or displacing drug). All drugs were dissolved in assay buffer at the concentration of 10⁻³ m with the following exception: ketanserin was dissolved in water and/or acid, if necessary. After an incubation of 30 min at 25 °C, assay mixtures were rapidly filtered through no. 32 glass fiber filters (Schleicher and Schuell) and washed 2 times with 5 ml of 50 mM Tris-HCl buffer (pH 7.8). The filters were transferred to plastic counting vials, and radioactivity was measured by liquid scintillation spectroscopy in 2.5 ml of Bio-Safe II scintillation mixture (Research Products International Corp., Mount Prospect, IL). Specific binding was defined as the excess over blanks taken in the presence of 10⁻⁸ m 5-HT. The radioligand saturation data were analyzed by the EBDA (19) and LIGAND (20) programs that utilize the non-linear least squares curve fitting technique with the Marquardt-Levenberg modification of the Gauss-Newton method.

Northern Blotting, RFLP, Chromosomal Location, and CA Repeats—Human and rat mRNAs from striatum, pituitary, hypothalamus, frontal cortex, and cerebellum were extracted, run on formaldehyde agarose gel, and blotted on nitrocellulose membrane as previously described (21). The blots were probed and washed with 2 x...
**The Human 5-HT₁B Receptor**

SSC, 0.1% SDS at 55 °C for 20 min and with 0.1 × SSC, 0.1% SDS and exposed to x-ray film overnight at −70 °C with intensifying screens.

A rodent-human somatic cell hybrid panel was prepared for chromosomal mapping, as described (22, 23), and Southern blot hybridization of the hybrid cell panel was carried out (14). To further assign the clone, labeled DNA was hybridized to a specific activity of 1.2 × 10⁶ cpm/μg with [³²P]UTP and [³²P]dATP using a multiprime labeling system (Amersham Corp.). In situ hybridization was performed using metaphase chromosomes as described (24).

To search for polymorphic CA/GT repeats, blots containing cloned DNA were hybridized at 37 °C in a 50% formamide hybridization solution with 3′-end labeled oligonucleotides containing 12 tandem CA repeats, washed for 1 h at 62 °C with 2 × SSC, and exposed at room temperature for 12 h.

**Animals and Tissue Preparation and In Situ Hybridization**

Histochemistry—Monkeys (three male, adult Rhesus) and rats (three male, adult Wistar) were sacrificed. Cryostat brain sections (20 μm) were thaw-mounted onto gelatin-coated slides and kept at −2 °C. Human tissues obtained at autopsy were provided by Prof. A. Provat, University of Basel, Switzerland. Results presented were obtained with tissue from at least three individuals. Oligonucleotides (48-mers) complementary to regions of the amino terminus, third cytoplasmic loop, and carboxyl terminus were synthesized and labeled as described (25). Prior to hybridization, frozen tissue sections were brought to room temperature, air-dried for 20 min, fixed by immersion in 4% paraformaldehyde in phosphate-buffered saline (PBS) 2.6 mM KCl, 14.5 mM NaCl, 0.5 mM Na₂HPO₄, 0.8 mM KH₂PO₄, 10 mM NaOH, pH 7.5, and washed once in 3 × PBS, twice in 1 × PBS, 5 min each, and incubated in a fresh solution of predigested Pronase at a final concentration of 24 μg/ml in 50 mM Tris-HCl, pH 7.5, 5 mM EDTA for 10 min at room temperature. Protoprotective activity was stopped by immersion for 30 s in 2 mg/ml glycine in PBS. Tissues were rinsed in PBS, dehydrated in a graded series of ethanol (60, 70, 80, and 100% each), and air-dried at −70 °C with intensifying screens for 1 month. Bright field photomicrographs were taken after staining the tissue with 2% Giemsa/PBS solution.

**RESULTS AND DISCUSSION**

The Nucleotide Sequencing of the Putative Receptor Gene, HJ-1—Genomic DNA was amplified in the PCR with a pair of degenerate oligonucleotides (Fig. 1 (legend)), based on the sequence encoding TM3 and TM6 of three serotonin receptors. Colonies were probed with a gene fragment which encodes the 5-HT₁A receptor (4). Twenty-six probe binding colonies were obtained, and six were picked and sequenced. They encoded TM3 to TM6 of 5-HT₁A receptor.

Thirty-six non-probe binding colonies were sequenced. The sequences could be classified into three categories: clone 35 encoded the D5 dopamine receptor gene; clones 23, 30, and 34 were identical and encoded TM3 to TM6 of the recently cloned 5-HT₁D receptor, and clone 27 was also, but to a less extent, homologous to 5-HT₁D. The full gene encoded by clone 27, a human genomic library was screened, and two positive clones were obtained. A 2-kb SacI fragment from each of the clones was found to hybridize to a 0.6-kb fragment of clone 27, and these were subcloned into Bluescript plasmid and sequenced. The restriction map (Fig. 1A) and sequence of each clone were determined and found to be identical. The clone, initially called HJ-1, contained an intronless open reading frame of 1170 nucleotides, encoding a 390-amino acid protein (Fig. 1B). The deduced amino acid sequence possesses characters of the G-protein-coupled receptor genes (26). Southern blot analysis of human genomic DNA, cut with either PstI, BgIII, or SacI and probed with HJ-1, revealed the presence of a single hybridizing band with each enzyme (data not shown).

Like the 5-HT₁D receptor the protein encoded by the HJ-1 gene has a relatively long third intracellular loop and a short carboxyl-terminal cytoplasmic tail, a feature common to

![Figure 1](image-url)
The Human 5-HT₁B Receptor

5-HT₁B receptors, that are linked to inhibition of adenylcyclase (26). The amino acid sequence encoded by the HJ-1 gene has 68% overall homology and 77% similarity in transmembrane areas with the 5-HT₁B receptor. The percentage of amino acids encoded by this receptor that are identical with several other 5-HT receptors, both overall for the entire protein and within the transmembranous regions, are as follows: 44 and 56% with 5-HT₁A receptor, 27 and 38% with 5-HT₁C receptor, 28 and 35% with 5-HT₂ receptor.

Saturation Analysis of [³H]5-HT Binding to Human 5-HT₁B Receptors—A 1.4-kb NcoI/SalI fragment from HJ-1 was subcloned into the mammalian expression vector pRK (17, 18). This plasmid was used in the transient expression in 293 human kidney cell lines and showed that HJ-1 gene encoded a serotonergic receptor, which we have called the 5-HT₁B receptor.

Increasing concentrations of [³H]5-HT were used to analyze the saturation properties of expressed human 5-HT₁B receptors in the transfected cell line. Scatchard analysis of the data indicates that [³H]5-HT binding is monophasic over the concentration range analyzed. The \( K_D \) of [³H]5-HT is 16 ± 2 nM (\( n = 3 \)) while the \( B_{max} \) value of [³H]5-HT-labeled sites is 7800 pmol/g of protein (\( n = 3 \)). As shown in Table I, the drug affinity values obtained in this human 5-HT₁B receptor cell line are similar but not identical to the pharmacological analysis of drug interactions with expressed [³H]5-HT-labeled human 5-HT₁B receptors (8).

Tissue Distribution—Northern blot analysis revealed two mRNA transcripts in human tissue, both of which were abundant in striatum, moderately expressed in hippocampus and frontal cortex, and barely detectable in pituitary and cerebellum (Fig. 2). The differently sized transcripts may result from the use of alternative transcription start sites. A rat Northern blot showed the same distribution pattern, and 5-HT₁B mRNA was expressed 6–8 times more in striatum (as indexed by scanning densitometry) than in other brain areas (Fig. 2). 5-HT₁B mRNA could not be detected in tissues from rat liver, heart, kidney, lung, spleen, or in the human neuroblastoma cell line SK-N-MC (ATCC, data not shown).

Our in situ hybridization experiments (Fig. 3) show that 5-HT₁B transcripts are present in the mammalian brain in a widespread but heterogeneous manner. The distribution is in agreement with that found by Northern blot assays. Fig. 3 illustrates the distribution of 5-HT₁B transcripts; in the three species examined the nucleus caudatus and putamen presented important levels of hybridization signal that were homogeneously distributed. Signals were highest in the rat brain and lower in human and Rhesus monkey brain. In addition, a band with high levels of hybridization (corresponding to lamina V) was observed in neocortical areas in all three species. Finally, the cerebellar cortex showed hybridization, particularly in the Purkinje cell layer. In the rat brain very high levels of hybridization signal were also observed in the pyramidal cell layer of the CA field and several thalamic nuclei. The enrichment of 5-HT₁B transcripts in the basal ganglia is in good agreement with the presence of high densities of [³H]5-HT binding sites in these brain regions and in the areas receiving afferents from the basal ganglia, i.e. the globus pallidus and substantia nigra pars reticulata (27). The

![Figure 2](image_url)

**FIG. 2.** Northern blot analysis of the tissue distribution of the 5-HT₁B receptor mRNA. Each lane contains 10 µg of polyadenylated RNA from various regions of human (A) and rat (B) brain.

**Table I**

| Drug                | \( K_D \) (nM) | \( B_{max} \) (pmol/g protein) | Ratio 5-HT₁B/5-HT₁A |
|---------------------|----------------|--------------------------------|---------------------|
| 5-HT                | 17 ± 2         | 8.9                            | 2                   |
| Methiothepin        | 17 ± 2         | 5.8                            | 3                   |
| Dihydroergotamine   | 18 ± 8         | 0.13                           | 138                 |
| Sumatriptan         | 61 ± 5         | 3.7                            | 17                  |
| Pindolol            | 17,000 ± 2,000 | 300                            | 9                   |
| 8-OH-DPAT           | 2,600 ± 100    | 8,100                          | 9                   |
| Ketanserin          | 8,100 ± 1,000  | 100                            | 100                 |
| Propranolol         | 14,000 ± 300   | 300                            | 9                   |

* 5-Carboxyamidotryptamine.
* 8-Hydroxy-2-(di-n-propylamino)tetrailin.

![Figure 3](image_url)

**FIG. 3.** Localization of the 5-HT₁B receptor in human (panels A and B), monkey (panels C and D), and rat (panels E and F) brain by in situ hybridization histochemistry. Cd, caudate; Cx, cortex; Put, putamen; Hp, hippocampus. Bars, 5 mm.
distribution of 5-HT₁B transcripts is clearly different from that of 5-HT₁A, 5-HT₁C, and 5-HT₂ mRNAs (28), but a certain degree of overlap exists with that of RDC4 mRNA in the same species not only in the basal ganglia but also in the dorsal raphe is present. This is an important observation in view of the heterogeneous nature of 5-HT binding sites in these brain regions (28).

**Chromosomal Location of 5-HT₁B and 5-HT₁D—**Chromosomal location of the 5-HT₁B and 5-HT₁D receptor genes were determined by Southern blot analysis of DNAs from a rodent-human hybrid cell panel. Chromosome 6 was revealed to be the only human chromosome that segregated perfectly with the 5-HT₁B gene; and chromosome 1 was shown to be the only human chromosome that segregated perfectly with the 5-HT₁D gene fragment; every other human chromosome could be excluded by two or more discordant hybrids (data not shown). The analysis of the distribution of 200 silver grains following in situ hybridization with HJ-1 revealed a significant clustering of 38 grains in the 6cen-6q14 region, with a maximum peak in the 6q13 region. Genomic DNAs from five unrelated individuals were found not to be polymorphic for either 5-HT₁B or 5-HT₁D, with the following enzymes: PstI, PvuII, BamHI, BglII, EcoRI, TaqI, XbaI, BglII, SstI, Rsal, HindIII, and MspI.

Phage clones containing the receptors 5-HT₁B and 5-HT₁D, inserts of approximately 15 kb of genomic DNA, were digested with several enzymes, electrophoresed along with a positive control containing a CA/GT repeat, and Southern-blotted according to standard techniques. The results indicate the absence of these repeats at the general vicinity of the two serotonergic receptor genes.

Prior to the present report, 5-HT₁B receptor binding sites were felt to be localized to the brains of certain rodents (29, 30). The reason that the human 5-HT₁B receptor binding site could not be identified previously in human brain relates to the fact that it is pharmacologically similar, but not identical, to the human 5-HT₁D receptor (6). The unequivocal differentiation, by molecular biological techniques, of human 5-HT₁B receptors and human 5-HT₁D receptors (8) confirms and significantly extends these earlier observations.

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**REFERENCES**

1. Osborne, N. N. (1982) in *Biological of Serotonergic Transmission* (Osborne, N. N., ed) pp. 7-27, John Wiley and Sons Ltd., Chichester, United Kingdom.
2. Vogt, M. (1983) in *Biological of Serotonergic Transmission* (Osborne, N. N., ed) pp. 292-315, John Wiley and Sons Ltd., Chichester, United Kingdom.
3. Caron, M. G. and Sigel, L. H. A. (1986) *Ann. Rev. Neurosci.* 9, 61-80.
4. Kohlika, B. K., Thomas, F., Collins, S., Yang-Feng, T., Kohlika, T. S., Francke, U., Leftkowitz, R. J., and Caron, M. G. (1987) *Nature* 326, 75-79.
5. Futini, A., Raymond, J. R., Lohnes, M. J., Kohlika, B. K., Caron, M. G., and Lefkowitz, R. J. (1988) *Nature* 335, 358-360.
6. Julian, D., MacDermott, A. B., Axel, R., and Jessell, T. M. (1988) *Science* 241, 558-564.
7. Fritchel, D. B., Bach, A. W. J., Wozny, M., Taleb, O., Da Tose, R., Shiib, J. C., and Seeburg, P. H. (1986) *EMBO J.* 5, 4136-4140.
8. Hamblin, M. W., and McCall, M. A. (1991) Mol. Pharmacol. 40, 143-148.
9. Julian, D. (1991) *Ann. Rev. Neurosci.* 14, 335-360.
10. Hartig, P., Kao, H. T., Macchi, M., Adham, N., Zgombick, J., Weinhandl, S., and Rache, T. (1990) *Neuropsychopharmacology* 3, 355-347.
11. Saiti, T. K., Gefend, D. H., Stoffel, S. E., Schert, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A. (1988) *Science* 239, 487-491.
12. Leibert, F., Parnatt, M., Lefort, A., Dinsfort, C., Van Sande, J., Mazzaab, C., Simon, M., Dumont, J. E., and Vassart, G. (1988) *Science* 244, 568-572.
13. Nguyen, T., Bard, J., Jin, H., Tanucio, D., Ward, D. C., Kennedy, J. L., Weinhandl, R., Seeman, P., and O'Dowd, B. F. (1991) *Gene* (Amst.) 109, 211-218.
14. Maurj, T., Fritsch, E. F., and SAMbrok (1999) *Molecular Cloning: A Laboratory Manual, pp. 10.6-10.12, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
15. Ausbel, P. M., Brent, P., Kingston, R. M., Moore, D. D. Seidman, J. G., Smith, J. A.: and Stedman, K. (1979) Proc. Natl. Acad. Sci. U. S. 76, 7373-7376.
16. Eton, D. L., Wood, W., Eton, D., Hass, P., Hollonsghead, P., Wion, K., Mahler, J., Vehar, J., and Corman, C. (1986) *Biochemistry* 26, 8343-8347.
17. McPherson, G. A. (1983) *Comput. Programs Biomed.* 17, 197-114.
18. Manion, P. J., and Rodbard, D. (1980) *Anal. Biochem.* 717, 229-239.
19. Sunahara, R. K., Guan, H. C., O'Dowd, B. F., Seeman, P., Laurier, L. G., Ng, G., George, S. R., Torchia, J., Van Tol, H. H. M., and Namik, H. B. (1991) *Nature* 350, 614-619.
20. Baas, F., Blik, H., Van Ommen, G. J. B. M., and Vijlder, J. M. (1984) *Hum. Genet.* 67, 361-366.
21. de Martinville, B., Wyman, A. R., White, R., and Frank, U. (1982) Am. J. Hum. Genet. 34, 216-226.
22. Duncan, A. M. V., Buchwald, M., and Tsai, L-C. (1986) *Cyto genetic Cell* 49, 369-310.
23. Menged, G., Villaro, M. T., Namik, H. B., Sunahara, R. K. Seeman, P., Namik, H. B., and Namik, H. B. (1989) *Mol. Brain Res.* 16, 195-191.
24. O'Dowd, B., Hnatowich, M., and Lefkowitz, R. J. (1991) *Encyclopedia of Human Biology, Vol. 1, pp. 81-92, Academic Press, San Diego.
25. Palacios, J. M., Waeber, C., Menged, G., and Pompeiano, M. (1991) in *Serotonon: Molecular Biology, Receptors and Functional Effects* (Forzard, P. J., and Saxena, P. R., eds), pp. 8-20, Birkhauer, Basel.
26. Palacios, J. M., Waeber, C., Menged, G., and Pompeiano, M. (1991) in *Serotonon: Molecular Biology, Receptors and Functional Effects* (Forzard, P. J., and Saxena, P. R., eds), pp. 107-116, Birkhauer, Basel.
27. Humin, R. E., and Peroutka, S. J. (1987) *Neurosci.* 7, 894-903.
28. Hoven, D., and Mullem, D. (1989) *Trends Pharmcol. Sci.* 10, 130-132.