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Mouse 5HT1B serotonin receptor: Cloning, functional expression, and localization in motor control centers

(cyclic AMP/guanine nucleotide-binding protein/striatum/cerebellum/Purkinje cells)

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ABSTRACT **Serotonin is a neuromodulator that mediates a wide range of effects by interacting with multiple receptors. Using a strategy based on nucleotide sequence homology between genes encoding receptors that interact with guanine nucleotide-binding proteins, we have isolated a mouse gene encoding an additional serotonin receptor. When expressed in cultured cells, it displayed the pharmacological profile and coupling with adenylate cyclase characteristic of the 5HT1B receptor subtype. In NIH 3T3 cells expressing this receptor, serotonin induced a decrease in forskolin-stimulated cAMP levels. This effect was blocked by pertussis toxin, indicating that the 5HT1B receptor interacts with a pertussis toxin-sensitive guanine nucleotide-binding protein. To obtain clues as to the possible function of the 5HT1B receptor, we have analyzed its pattern of expression in the adult mouse brain by *in situ* hybridization. Our results, together with previous autoradiographic studies, suggest that the 5HT1B receptors are localized presynaptically on the terminals of striatal neurons and Purkinje cells and that they might modulate the release of neurotransmitters such as γ-aminobutyric acid. The predominant expression of the 5HT1B receptor in the striatum and cerebellum points to an involvement of this receptor in motor control.**

Serotonin (5-HT) is a neuromodulator that is involved in various functions such as sleep, appetite, pain perception, and vascular contraction. This diversity of effects can be related to the fact that the 5-HT-ergic neurons project into virtually all parts of the brain and spinal cord, although their cell bodies are concentrated in a limited area, the raphe nuclei. 5-HT activates multiple receptor subtypes that exhibit distinct pharmacological properties, signaling systems, and tissue distributions (for a review, see ref. 1). The 5HT1B receptors have been identified in the rat and mouse brain where their highest density was found within the globus pallidus and the substantia nigra. However, they could not be detected in the brain of other species, including humans. These species contained, instead, 5HT1D receptors that have a slightly different pharmacological profile but the same tissue distribution. It was therefore suggested that the 5HT1B and 1D receptors correspond to species variants of a same receptor subtype. The 5HT1B and the 5HT1D receptors are negatively coupled with adenylate cyclase. Recent cloning of the 5HT1A, 5HT1C, and 5HT2 receptors has revealed that they belong to the large family of receptors that interact with guanine nucleotide-binding proteins (G proteins) and share a predicted seven-transmembrane-domain structure (2). We have exploited the sequence homologies that exist between several members of this family to clone the gene encoding the mouse 5HT1B receptor.† Our results suggest that the 5HT1B receptors are localized presynaptically on the terminals of striatal neurons and Purkinje cells.

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MATERIAL AND METHODS

Isolation and Sequence of the 5HT1B Genomic Clone. A nested PCR experiment was performed on mouse genomic DNA with the following oligonucleotides: (*i*) TACCTCGAGGTCGACGGTITG(C/T)TGG(C/T)TICCITT(C/T)TT; (*ii*) AGAACTAGTGGTACCC(G/A)TIGT(G/A)TA(G/A/T)ATIA(C/T)IGG(G/A)TT; (*iii*) AGAACTAGTGGTACCC(G/C)(T/A)(G/A)TTIAC(G/A)TAICCIA(A/G)CCA. One microgram of DNA was annealed at 55°C and amplified at 72°C in the presence of 3 mM $MgCl_2$ for 20 cycles with primers *i* and *ii* and for 20 more cycles with primers *i* and *iii*. The PCR products were cut with *Xho* I and *Kpn* I cloned in the Bluescript plasmid and sequenced. One of the deduced amino acid sequences resembled that of 5-HT-ergic receptors. The corresponding oligonucleotides were synthesized (TGGCCATGTGAAACCAGCAGGCATC; TTCCCTGGTGATGCCTATCGTAAG) and used to screen a mouse genomic library. The *Bgl* II–*Sac* I fragment (Fig. 1) hybridizing with these two oligonucleotides was sequenced on both strands by the dideoxynucleotide technique using successive synthetic oligonucleotides.

Expression of the 5HT1B Receptor in Cultured Cells. The *Bgl* II–*Sac* I genomic fragment (Fig. 1) was inserted into the *Bgl* II and *Sac* I sites of expression vector p513, which is a derivative of pSG5 (3) containing a multiple cloning site. The resulting recombinant was introduced into mouse NIH 3T3 cells by calcium phosphate-mediated transfection together with the recombinant pRSVneo, which encodes resistance to G418 (20 μg of 5HT1B recombinant and 1 μg of pRSVneo per 10-cm dish). Transformed clones were selected in the presence of 0.5 mg of G418 per ml. Isolated foci were amplified and total RNA was prepared and analyzed for expression of 5HT1B mRNA. Two cell lines were selected that expressed high levels of 5HT1B mRNA as determined by Northern blot analysis.

For transient expression of the 5HT1B receptor, COS-7 cells were transfected by the calcium phosphate technique with the 5HT1B recombinant alone (20 μg per 10-cm dish) and analyzed 48 hr after transfection.

Radioligand Binding Assays. Membranes were prepared (4) and [^{3}H]5-HT binding assays and competition displacement experiments were performed as described (5).

cAMP Assays. Cells were seeded into 12-well plates at a density of ≈3 × 10^5 cells per well, washed once with phosphate-buffered saline (PBS), and incubated for 15 min at

Abbreviations: G protein, guanine nucleotide-binding protein; 5-HT, 5-hydroxytryptamine.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M85151).

37°C with 100 μM isobutylmethylxanthine and test agents in PBS. The reaction was stopped by aspiration of the medium followed by addition of 500 μl of ice-cold ethanol. After 2 hr at room temperature, the ethanol was collected and lyophilized. The pellet was reconstituted and cAMP was quantitated using a radioimmunoassay kit (NEN, NEK-033). The basal level of cAMP observed in the absence of drugs was about the same in all cell lines (≈300 pmol/mg of protein). One micromolar forskolin typically yielded a 10-fold increase in cAMP levels.

RNA Analysis. Poly(A)$^+$ mRNA was prepared, fractionated on a 1% agarose/formaldehyde gel, and transferred to a nitrocellulose filter. The DNA probe was the *Bgl* II–*Sac* I fragment, which was ^{32}P-labeled by random priming and hybridized to filters at high stringency [42°C; 50% formamide/5 × SSC (1 × SSC = 0.15 M NaCl/15 mM sodium citrate)/1 × Denhardt's solution/20 mM sodium phosphate buffer, pH 6.5/0.1% SDS/100 μg of tRNA per ml]. Washings were performed at high stringency (60°C; 0.1 × SSC/0.1% SDS).

***In Situ* Hybridization.** *In situ* hybridizations were performed on cryostat sections of adult mouse brains (about 8 weeks old) as described (6). The probe used was the 5HT1B *Bgl* II–*Sac* I genomic fragment labeled by random priming with ATP[^{35}S]. Slides were exposed for 10 days.

RESULTS

Isolation of a Mouse Genomic Clone Encoding an Additional Member of the G Protein-Coupled Receptor Family. Sequence comparisons of G protein-coupled receptors have revealed a striking amino acid sequence conservation, particularly in certain putative transmembrane domains such as domains VI and VII. We decided therefore to use degenerate oligonucleotides corresponding to these two regions to perform a series of PCR experiments on mouse genomic DNA. The resulting fragments were subcloned and sequenced. One of these fragments was used to screen a mouse genomic library. We obtained two phage recombinants that contained a 2.3-kilobase-pair-long *Bgl* II–*Sac* I fragment (Fig. 1) hybridizing with the PCR product. Sequence analysis of this fragment revealed one long open reading frame encoding a predicted protein that exhibited highest homology to the human 5HT1D receptor (59%) (7) and to the rat 5HT1A receptor (47%) (8).

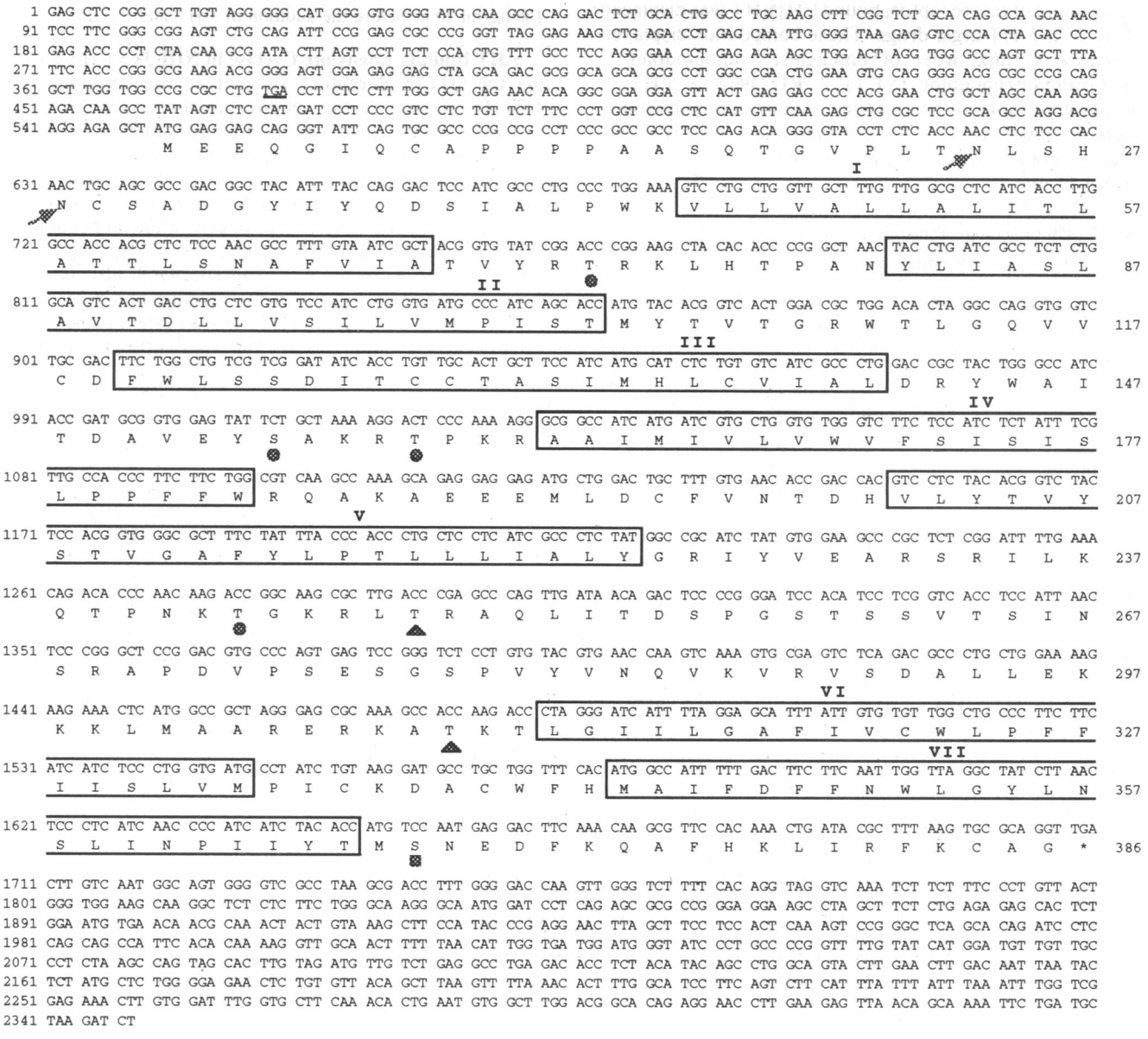

FIG. 1. Nucleotide sequence of the *Sac* I–*Bgl* II genomic fragment encoding the 5HT1B receptor. The seven putative transmembrane domains are boxed and numbered (I–VII). Arrows indicate sites of potential N-linked glycosylation. ●, ▲, and ■, consensus sites for phosphorylation by protein kinase C, protein kinase A, and tyrosine kinase, respectively.

Hydropathy analysis of this predicted protein revealed seven hydrophobic domains (numbered I–VII in Fig. 1), a feature shared by all other cloned members of the G protein-coupled receptor family. The amino-terminal end contained two putative sites for N-linked glycosylation and the presumed cytoplasmic domains contained several consensus sites for phosphorylation by protein kinases C and A (Fig. 1).

The Mouse Receptor Has the Same Pharmacological Profile as the 5HT1B Receptor. To determine whether the genomic fragment that we had isolated 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 5-HT-ergic radioligands. Although ^{125}I-labeled lysergic acid diethylamide, ^{3}H-labeled ketanserine, and ^{3}H-labeled 8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT) did not bind to these membranes, [^{3}H]5-HT displayed a single saturable binding site: K_d = 48 nM and B_{max} = 19 pmol/mg of membrane protein (not shown). In a control experiment, [^{3}H]5-HT did not bind to mock-transfected COS-7 cells. To determine the pharmacological profile of this receptor, bound [^{3}H]5-HT was displaced with various 5-HT-ergic drugs. These compounds displayed the following rank order of potencies: cyanopindolol > 5-carboxamidotryptamine (5-CT) = RU24969 > 5-HT > (−)-pindolol (Table 1). Ketanserine, mianserine, yohimbine, spiperone, and 8-OH-DPAT were almost inactive. This profile corresponds well with that of the rat brain 5HT1B receptor (Table 1).

Table 1. Pharmacological profile of the 5HT1B receptor

Drug	Binding, pK_d'		Cyclase, pEC_{50} or pK_i	
	Mouse COS-7 cells	Rat cortex	Mouse NIH 3T3 cells	Rat substantia nigra
Agonist				
5-HT	7.4 (4)	7.6	7.8 (3)	7.8
5-CT	8.0 (3)	8.3	8.1 (2)	7.9
RU24969	8.0 (3)	8.4	8.3 (2)	8.4
Antagonist				
Cyanopindolol	8.6 (3)	8.3	8.8 (3)	8.2
Methiothepin	—	7.3	8.4 (2)	8.1
(−)-Pindolol	7.2 (2)	7.2	—	6.8
Mianserine	5.4 (2)	5.3	—	5.9
Ketanserine	5.2 (2)	—	—	—
Yohimbine	4.7 (2)	5.5	—	6.1
Spiperone	4.6 (2)	5.3	—	4.4
8-OH-DPAT	4.5 (2)	4.2	—	4.8

Binding data correspond to competition for [^{3}H]5-HT binding to membranes of COS-7 cells expressing the mouse 5HT1B receptor. IC_{50} values required to displace 50% of [^{3}H]5-HT were determined experimentally and converted to pK_d' values according to the equation $K_d' = IC_{50}/(1 + C/K_d)$, where C is the [^{3}H]5-HT concentration (25 nM) and K_d is the equilibrium dissociation constant of [^{3}H]5-HT (48 nM). Cyclase data were obtained with an NIH 3T3-derived cell line expressing the mouse 5HT1B receptor. EC_{50} is the concentration of agonist required to obtain a half-maximal inhibition of forskolin-stimulated adenylate cyclase. The concentrations of antagonist required to inhibit 5-HT's effect by 50% (IC_{50}) were determined and converted to pK_i values according to the equation $K_i = IC_{50}/(1 + C/K_d)$, where C is the 5-HT concentration (100 nM) and K_d is the EC_{50} value for 5-HT (14 nM). Binding and cyclase data were compared to those reported in rat cortex and rat substantia nigra (9). Numbers in parentheses correspond to the number of independent experiments performed. The values presented are the mean of at least two independent experiments (each determination performed in triplicate). Individual K_d', EC_{50}, and K_i values differed by <20%. 5-CT, 5-carboxamidotryptamine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin.

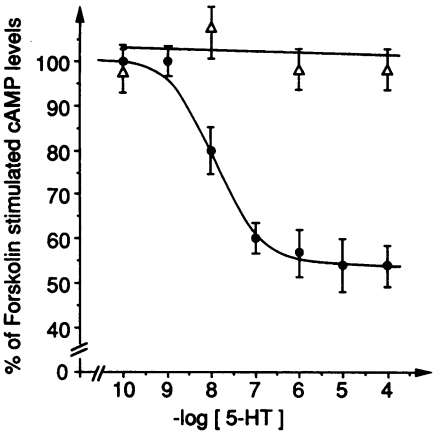

FIG. 2. 5-HT induced a decrease in cAMP levels in NIH 3T3 cells expressing the 5HT1B receptor. cAMP levels were expressed as a percentage of the value obtained with 1 μM forskolin (100%). Data are the mean of three independent experiments (each determination performed in triplicate). ●, 5-HT plus forskolin; △, 5-HT, forskolin, and pertussis toxin. Pertussis toxin was applied at a concentration of 100 ng/ml 20 hr before the addition of 5-HT and forskolin.

5-HT Inhibits Adenylate Cyclase in NIH 3T3 Cells Expressing the 5HT1B Receptor. To analyze the coupling of the 5HT1B receptor to the second messenger machinery, we generated stable clonal cell lines expressing this receptor. In two independent cell lines expressing high levels of 5HT1B mRNA, 5-HT mediated a decrease in forskolin-stimulated cAMP levels but had no effect on control NIH 3T3 cells. This decrease in cAMP level was concentration dependent and saturable, the EC_{50} for 5-HT being 1.4×10^{-8} M (Fig. 2). Two other 5HT1B agonists, 5-CT and RU24969, also induced a decrease in cAMP levels, with EC_{50} values of 8×10^{-9} M and 5×10^{-9} M, respectively (Table 1). The effect of 5-HT could be blocked by cyanopindolol and methiothepin, which are 5HT1B antagonists. The resulting EC_{50} and K_i values (Table 1) are in good agreement with those we obtained in binding assays and with the values reported in rat substantia nigra for the 5HT1B receptor (9). Pertussis toxin blocked the effect of serotonin (Fig. 2), indicating that the 5HT1B receptor is coupled to a pertussis toxin-sensitive G protein.

The 5HT1B Receptor Is Expressed Predominantly in the Striatum and Cerebellum. Expression of 5HT1B transcripts was analyzed by Northern blot and *in situ* hybridization experiments. The Northern analysis revealed that expression was predominant in nervous tissue. A 6-kb transcript was detected in forebrain, hindbrain, cerebellum, and spinal cord (Fig. 3 and results not shown). To further analyze the pattern of expression of this receptor we performed *in situ* hybridization experiments on brain sections (Fig. 4). The main sites of expression were the caudate-putamen and the Purkinje

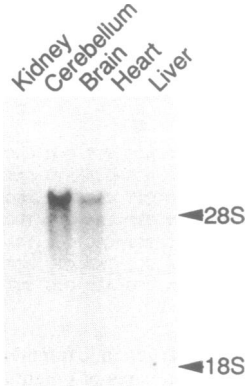

FIG. 3. Distribution of 5HT1B transcripts. Northern blot analysis of poly(A)$^+$ RNA (5 μg) from various organs. A 6-kb RNA was detected in cerebellum and total brain and detected very faintly in kidney. The probe used was the ^{32}P-labeled *Sac* I–*Bgl* II fragment.

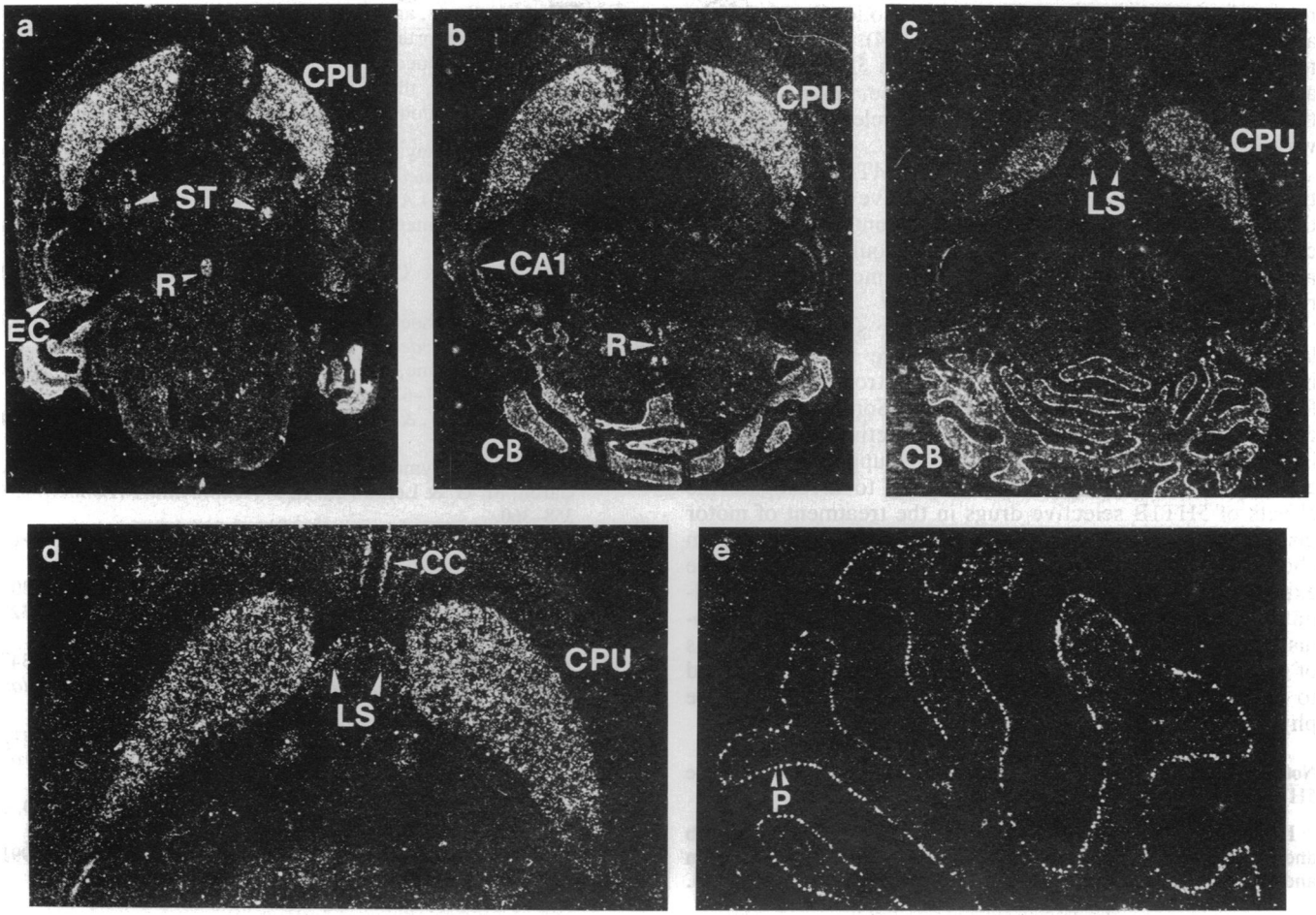

FIG. 4. *In situ* hybridization to mouse brain horizontal sections. (*a–c*) Dark-field pictures or successively more dorsal sections of a whole brain (8 mm wide). (*d*) A 1.5-fold magnification (compared to *a–c*) of the striatal region at a depth intermediate between that of *b* and *c*. (*e*) A 4-fold magnification (compared to *a–c*) of a cerebellar region at about the same depth as *c*. CPU, caudate-putamen; CB, cerebellum; CC, cingulate cortex; CA1, hippocampal area; EC, entorhinal cortex; LS, lateral septum; P, Purkinje cells; R, raphe nuclei; ST, subthalamic nuclei.

cells of the cerebellum. Weaker signals were also detected in the hippocampus, raphe nuclei, lateral septum, subthalamic nuclei, cingulate cortex, and entorhinal cortex. In a control experiment performed under the same conditions with plasmid DNA fragments instead of the 5HT1B genomic fragment, no hybridization was observed.

DISCUSSION

Our binding and cyclase data indicate that the genomic clone that we have isolated encodes a functional mouse 5HT1B receptor. This receptor exhibits 59% amino acid identity with the recently cloned human 5HT1D receptor (7). However, it is unlikely that "our 5HT1B receptor" is the mouse counterpart of that 5HT1D receptor, because, in the cases known so far, receptor subtypes exhibit a higher degree of conservation across mammalian species—usually 85–95% amino acid identity. Furthermore, we have recently cloned another mouse receptor that is 89% homologous to the cloned 5HT1D receptor (results not shown) and that is therefore most likely the mouse counterpart of the 5HT1D receptor. Our results suggest therefore that the 5HT1B/5HT1D receptors constitute a heterogenous family consisting of at least two different receptors in each mammalian species.

The pattern of expression of the 5HT1B mRNA indicates that a large proportion of the 5HT1B receptors are located postsynaptically with respect to the afferent 5-HT-ergic fibers originating in the raphe nuclei. Only the transcripts expressed in the raphe nuclei (Fig. 4 *a* and *b*) corresponds to receptors localized on the 5-HT-ergic neurons or autoreceptors. Such autoreceptors have been proposed to modulate the release of 5-HT from the terminals of 5-HT-ergic neurons (for a review, see ref. 1).

The main sites of expression of 5HT1B mRNA are the striatum (caudate-putamen) and the Purkinje cells of the cerebellum (Fig. 4). The comparison between this mRNA pattern and the pattern of the 5HT1B binding sites determined by autoradiography is indicative of the pre- or postsynaptic localization of this receptor. The 5HT1B binding sites were found mostly in the globus pallidus and substantia nigra (10) and to a lesser extent in the caudate-putamen. In contrast, 5HT1B transcripts were detected in the caudate-putamen but not in the globus pallidus and substantia nigra. Since most efferent fibers from the caudate-putamen project to the globus pallidus and substantia nigra, it is likely that the 5HT1B receptors present in the globus pallidus and substantia nigra are localized presynaptically on the terminals of projecting striatal neurons. In good agreement with this hypothesis is the observation that experimental or pathological lesions of the caudate-putamen, such as those observed in Huntington chorea (11, 12), result in a decrease in 5HT1B/1D sites not only in the caudate-putamen but also in the globus pallidus and substantia nigra. The presynaptic localization of these 5HT1B receptors suggests that they might modulate the release of neurotransmitters such as γ-aminobutyric acid (GABA), substance P, or enkephalins from striatal neuron terminals. Interestingly, the dopamine D1 receptors that have been reported to stimulate GABA

release in the substantia nigra (13) are also localized presynaptically on projecting striatal neurons (14). Since D1 receptors activate adenylate cyclase, whereas 5HT1B receptors are negatively coupled to adenylate cyclase, it is possible that these two receptors modulate GABA release in opposite ways in the substantia nigra.

The other main site of expression of 5HT1B transcripts is the Purkinje cells. 5HT1B binding sites have been detected in the deep nuclei of the cerebellum that contain the Purkinje cell terminals (10). These 5HT1B sites could therefore correspond again to presynaptic receptors modulating GABA release from Purkinje cells.

Expression of 5HT1B receptors in the striatum, subthalamic nuclei and cerebellum, which are brain structures involved predominantly in movement control, suggests a role for these receptors in motor function. Some experimental data, such as an increase in locomotor activity after administration of 5HT1B agonists to rats (15), support this hypothesis. Therefore, it might be interesting to investigate the effects of 5HT1B selective drugs in the treatment of motor disorders of the striatum and cerebellum, such as Huntington chorea and cerebellar ataxias. The availability of a cell line expressing high levels of this receptor subtype should facilitate the development of new 5HT1B agonists and antagonists. The 5HT1B genomic clone will also allow us by means of gene-targeting techniques, to produce mouse mutants and to analyze the consequences of these mutations on the physiology of the animal.

Note Added in Proof. A rat 5HT1B receptor homologous to the mouse 5HT1B receptor has been recently described (16).

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1. Frazer, A., Maayani, S. & Wolfe, B. B. (1990) *Annu. Rev. Pharmacol. Toxicol.* **30,** 307–348.
2. Julius, D. (1991) *Annu. Rev. Neurosci.* **14,** 335–360.
3. Green, S., Isseman, I. & Sheer, E. (1988) *Nucleic Acids Res.* **16,** 369–370.
4. Amlaiky, N. & Caron, M. G. (1985) *J. Biol. Chem.* **260,** 1983–1986.
5. Waeber, C., Schoeffter, P., Palacios, J. M. & Hoyer, D. (1989) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **340,** 479–485.
6. Hafen, E., Levine, M., Garber, R. L. & Gehring, W. J. (1983) *EMBO J.* **2,** 617–623.
7. Hamblin, M. W. & Metcalf, M. A. (1991) *Mol. Pharmacol.* **40,** 143–148.
8. Fargin, A., Raymond, J. R., Lohse, M. J., Kolbika, B. K., Caron, M. G. & Lefkowitz, R. J. (1988) *Nature (London)* **335,** 358–360.
9. Schoeffter, P. & Hoyer, D. (1989) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **340,** 285–292.
10. Pazos, A. & Palacios, J. M. (1985) *Brain Res.* **346,** 205–230.
11. Waeber, C., Zhang, L. & Palacios, J. M. (1990) *Brain Res.* **528,** 197–206.
12. Waeber, C. & Palacios, J. M. (1989) *Neuroscience* **32,** 337–347.
13. Reubi, J. C., Iversen, L. & Jessel, T. (1977) *Nature (London)* **258,** 652–654.
14. Fremeau, R. T., Duncan, G. E., Fornaretto, M. G., Dearry, A., Gingrich, J. A., Breese, G. R. & Caron, M. G. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3772–3776.
15. Oberlander, C., Demassey, Y., Verdu, A., van de Velde, D. & Bardeley, C. (1987) *Eur. J. Pharmacol.* **139,** 205–214.
16. Voight, M. M., Laurie, J. L., Seeburg, P. H. & Bach, A. (1991) *EMBO J.* **10,** 4017–4023.