

A NOVEL RAT SEROTONIN (5-HT₆) RECEPTOR : MOLECULAR CLONING, LOCALIZATION AND STIMULATION OF cAMP ACCUMULATION

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Using a strategy based upon nucleotide sequence homology and starting from the sequence of the rat histamine H₂ receptor (Ruat et al., Biochem. Biophys. Res. Commun. 1991, 179, 1470-1478), we have cloned a rat cDNA encoding a functional serotonin receptor (5-HT₆). Its coding sequence corresponds to a glycoprotein of 436 amino acids displaying significant homology with other cloned monoaminergic receptors, e.g., various serotonin receptors. Genomic analysis of its gene indicated the presence of at least one intron. The major transcript of the 5-HT₆ receptor gene has a size of ~4.1 kb but another minor 3.2 kb transcript was also evidenced. The highest expression, detected by Northern blot analysis as well as by *in situ* hybridization occurs in various serotonergic areas of rat or guinea pig brain such as striatum, olfactory tubercle, nucleus accumbens and hippocampus, but a faint expression is also detectable in rat stomach.

When transiently expressed in transfected COS-7 cells the 5-HT₆ receptor appears to be positively coupled to cyclic AMP production. © 1993 Academic Press, Inc.

Serotonin (5-HT) is a transmitter, namely released from cerebral and gastrointestinal neurons, which affects its target cells via interaction with a large variety of receptors recently defined by cloning of their genes (reviewed in refs. 1-4). There is evidence, however, for the existence of additional 5-HT receptors. For instance, whereas the cloned receptors appear to be coupled either positively to phospholipase C (those of the 5-HT₂ subfamily) or negatively to adenylyl cyclase (those of 5-HT₁ subfamily), there is evidence for 5-HT receptors stimulating adenylyl cyclase (5-9) or activating [³H]glycogen hydrolysis (10).

Using a nucleotide probe derived from the rat histamine H₂ receptor (11) to screen a cDNA library from rat striatum, we have characterized an additional 5-HT receptor that belongs

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to the superfamily of G protein-coupled receptors. Taking into account the recently proposed nomenclature rules (12) as well as the existence of the not yet cloned 5-HT₄ receptor (9) and of the 5-HT₅ receptor (13) signaling system of which remains to be identified, we propose to name this novel receptor 5-HT₆.

MATERIALS AND METHODS

Library screening. A partial Sau3A cut rat genomic library (11) was screened (10⁶ phages) at low stringency with a ³²P-labeled DNA fragment corresponding to a coding region of the rat histamine H₂ receptor gene [nucleotides 10 to 644 (11)]. Nitrocellulose filters (BAS 85, Schleicher and Schuell) were hybridized at 42°C for 18 h in 25% formamide, 1 x Denhardt's solution, 4 x SSC (standard saline citrate), Tris-HCl buffer 8 mM pH 7.4, 100 µg/ml of yeast tRNA and 20 µg/ml of denatured salmon sperm DNA with the ³²P-probe (2 x 10⁶ dpm/ml) and then washed for 2 x 20 min at 42°C in 2 x SSC 0.1% SDS, then for 2 x 20 min at 42°C in 0.2 x SSC 0.1% SDS before autoradiography. One clone (λG7A) was shown to hybridize strongly with the ³²P-probe and was further purified and analyzed. Several overlapping reactive DNA fragments were further subcloned in M13mp18 (Boehringer) and both strands were sequenced by the dideoxy chain termination method (14). These fragments encoded an open reading frame of 238 amino acids starting from an initiating methionine. (Met¹ in Fig.1) and including a consensus sequence for an intron in the 3' end.

A ³²P-labeled DNA fragment (nucleotides 129 to 640 in Fig.1) was prepared by PCR (15) using λG7A DNA as a template. This probe (2 x 10⁶ dpm/ml) was used to screen under high stringency (40% formamide) a rat striatal cDNA library (2 x 10⁶ phages) constructed in λZAP II (Stratagene). Eleven clones were plaque purified and plasmids Bluescript KS(+) containing cDNA inserts were recovered using the helper phage R508 according to the manufacturer procedure.

After amplification, both strands of the cDNA inserts were directly sequenced (14) using alkali-denatured plasmid DNA as a template. Two overlapping clones λS7A19 and λS7A20 exhibited an open reading frame encoding a protein of 436 amino acids and containing the open reading frame found in λG7A. A stop codon TGA was found in the 3' end. Genomic nucleotide sequence found in λG7A (nucleotides -319 to 714 in Fig.1) was identical to nucleotide sequences found in λS7A19 and λS7A20.

Transient expression in COS-7 cells. A 1.8 kb HindIII-SacII DNA fragment comprising the full length coding sequence of λS7A20 was excised from the Bluescript plasmid and purified by Gene-clean II (Bio 101). It was ligated at the HindIII-BglII site of an expression vector derived from the pSVD₂ (16), using a SacII-BglII adaptor. Cos-7 cells (96 mm petri dishes) were transfected with 30 µg of pSV5-HT₆, the expression vector described above, using the calcium phosphate method according to the Stratagene protocol. Forty-eight hours later, cells were harvested, put into 96-well plates and incubated for 24 h at 37°C. Cells were washed twice (10 min at 37°C) and incubated for 15 min with the appropriate drugs in Dulbecco's modified Eagle's medium containing 0.1 mM isobutylmethylxanthine and 0.05% ascorbate. Intracellular cAMP levels were determined by radioimmunoassay (DuPont) as described (17).

Northern blot analysis. Poly(A)⁺ mRNAs from Wistar rats or Hartley guinea pigs were subjected to Northern blot analysis as described (17). Hybridization was carried out overnight at 42°C with a probe (15 x 10⁶ dpm/ml) corresponding to nucleotides 129-640 in Fig.1 and ³²P-labeled by nick translation. Blots were washed three times in 2 x SSC/0.1 % SDS at 42°C for 30 min and twice in 0.2 x SSC/0.1 % SDS at 42°C for 30 min. They were then exposed to X-ray films at -80°C for 4 days with two intensifying screens.

In situ hybridization. Brain sections were prepared and incubated essentially as described (18). For hybridization, each brain section was covered with a buffer (65 % formamide, 10 % dextran

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tagccaggaaccccccccccatcttatggcatccccgggtggccctattccatccagggtctctcatccagcccaagctaactttcattgactc -225
gtcacatcagtagccctccccaaactctttaccgagtagctccaggtggccctgcgtaggaggcaccctacaactcctccgactctttgaaatcgctgctgatgacct -113
aagaaccccgttttgccaatactactctaaggtagcagcttcttctctccttttgccttcaccctgtacctgcagtcaccatataccgctcttggtctcaaccagtcctcc -1
      * * * *
ATG GTT CCA GAG CCA GGC CCT GTC AAC AGT AGC ACC CCA GCC TGG GGT CCC GGG CCA CCG CCT GCT CCG GGG GGC AGC GGC TGG      84
Met Val Pro Glu Pro Gly Pro Val Asn Ser Ser Thr Pro Ala Trp Gly Pro Gly Pro Pro Ala Pro Gly Gly Ser Gly Trp      28
      TM1
GTG GCT GCC GCG CTG TGC GTG GTC ATC GTG CTG ACA GCA GCC GCC AAT TCG CTG CTG ATC GTG CTC ATT TGC ACG CAG CCC GCG      168
Val Ala Ala Ala Leu Cys Val Val Ile Val Leu Thr Ala Ala Ala Asn Ser Leu Leu Ile Val Leu Ile Cys Thr Gln Pro Ala      56
      TM2
CTG CGC AAC ACG TCT AAC TTC TTT CTG GTG TCG CTC TTC ACG TCG GAC TTG ATG GTG GGG TTG GTG GTG ATG CCC CCA GCC ATG      252
Leu Arg Asn Thr Ser Asn Phe Phe Leu Val Ser Leu Phe Thr Ser Asp Leu Met Val Gly Leu Val Val Met Pro Pro Ala Met      84
      TM3
CTG AAC GCG CTG TAT GGG CGC TGG GTG TTA GCT CGA GGC CTC TGT CTG CTT TGG ACT GCC TTC GAC GTG ATG TGC TGC AGC GCC      336
Leu Asn Ala Leu Tyr Gly Arg Trp Val Leu Ala Arg Gly Leu Cys Leu Leu Trp Thr Ala Phe Asp Val Met Cys Cys Ser Ala      112
      TM4
TCC ATC CTC AAC CTC TGC CTC ATC AGC CTG GAC CGC TAC CTG CTC ATC CTC TCG CCG CTG CGC TAC AAG CTG CGC ATG ACA GCC      420
Ser Ile Leu Asn Leu Cys Leu Ile Ser Leu Asp Arg Tyr Leu Leu Ile Leu Ser Pro Leu Arg Tyr Lys Leu Arg Met Thr Ala      140
      TM5
CCG CGA GCC CTG GCG CTC ATC CTG GGT GCC TGG AGC CTC GCG GCG CTT GCC TCC TTC CTA CCC CTC TTG CTG GGC TGG CAC GAA      504
Pro Arg Ala Leu Ala Leu Ile Leu Gly Ala Trp Ser Leu Ala Ala Leu Ala Ser Phe Leu Pro Leu Leu Leu Gly Trp His Glu      168
      TM6
CTG GGC AAA GCT CGA ACA CCT GCC CCT GGC CAG TGC CGC CTA TTG GCC AGC CTG CCT TTT GTC CTC GTG GCG TCC GGC GTC ACC      588
Leu Gly Lys Ala Arg Thr Pro Ala Pro Gly Gln Cys Arg Leu Leu Ala Ser Leu Pro Phe Val Leu Val Ala Ser Gly Val Thr      196
      TM7
TTT TTC CTG CCT TCG GGT GCC ATC TGC TTC ACC TAC TGC AGG ATC CTT CTG GCT GCC CGC AAG CAG GCG GTG CAA GTG GCC TCG      672
Phe Phe Leu Pro Ser Gly Ala Ile Cys Phe Thr Tyr Cys Arg Ile Leu Leu Ala Ala Arg Lys Gln Ala Val Gln Val Ala Ser      224
      TM8
CTC ACC ACG GGC ACG GCT GGC CAG GCC TTG GAA ACC TTG CAG GTG CCC AGG ACA CCA CGC CCA GGG ATG GAG TCC GCT GAC AGT      756
Leu Thr Thr Gly Thr Ala Gly Gln Ala Leu Glu Thr Leu Gln Val Pro Arg Thr Pro Arg Pro Gly Met Glu Ser Ala Asp Ser      252
      TM9
AGG CGT CTG GCC ACC AAG CAT AGC AGG AAG GCC TTG AAG GCC AGC CTG ACC CTG GGC ATC CTG CTG GGA ATG TTC TTT GTC ACC      840
Arg Arg Leu Ala Thr Lys His Ser Arg Lys Ala Leu Lys Ala Ser Leu Thr Leu Gly Ile Leu Leu Gly Met Phe Phe Val Thr      280
      TM10
TGG CTG CCC TTC TTT GTG GCC AAC ATA GCT CAG GCC GTG TGT GAC TGC ATC TCC CCA GGC CTC TTC GAT GTC CTC ACA TGG CTG      924
Trp Leu Pro Phe Phe Val Ala Asn Ile Ala Gln Ala Val Cys Asp Cys Ile Ser Pro Gly Leu Phe Asp Val Leu Thr Trp Leu      308
      TM11
GGG TAC TGT AAT AGC ACC ATG AAC CCT ATC ATC TAC CCG CTC TTT ATG CGG GAC TTC AAG AGG GCC CTG GGC AGG TTC CTG CCA      1008
Gly Tyr Cys Asn Ser Thr Met Asn Pro Ile Ile Tyr Pro Leu Phe Met Arg Asp Phe Lys Arg Ala Leu Gly Arg Phe Leu Pro      336
      TM12
TGC GTC CAC TGT CCC CCG GAG CAC CGG CCA GCC CTG CCT CCC CCT CCA TGT GGA CCT CTC ACA GCG GTG CCA GAC CAG GCC TCA      1092
Cys Val His Cys Pro Pro Glu His Arg Pro Ala Leu Pro Pro Pro Pro Cys Gly Pro Leu Thr Ala Val Pro Asp Gln Ala Ser      364
      TM13
GCC TGC AGC AGG TGC TGC CTC TGC CTC TGC CGC CAA ACT CAG ATT CAG ACT CCG CTT CAG GGG GCA CCT CGG GCC TGC AGC TCA      1176
Ala Cys Ser Arg Cys Cys Leu Cys Leu Cys Arg Gln Thr Gln Ile Gln Thr Pro Leu Gln Gly Ala Pro Arg Ala Cys Ser Ser      392
      TM14
CAG CCC AGC TTC TGC TGC CTG GAG AGG CCA CCC GGG ACC CCC CGC CAC CCA CCA GGG CCA CCA CTG TGG TCA ACT TCT TTG TCA      1260
Gln Pro Ser Phe Cys Cys Leu Glu Arg Pro Pro Gly Thr Pro Arg His Pro Pro Gly Pro Pro Leu Trp Ser Thr Ser Leu Ser      420
      TM15
CAG ACT CTG TGG AGC CTG AGA TAC GGC CGC ATC CAC TCA GTT CCC CCG tgaactgaccaggtcaagagctggccattggaggccacattcccgga      1355
Gln Thr Leu Trp Ser Leu Arg Tyr Gly Arg Ile His Ser Val Pro Pro      436
      TM16
gctctcagcccactctccctgagactaggaggtgtaggtctcctgagagtgctgaattgaggtatcctcagctagccccatcttctgctgaggtccttgcacctgagg      1467
ggtagtcagaaacatctctgtgggtactccagtgcaatgttgcttgtagtggtcgggagggggcggtctcagagccggccatttggttcagggtcagtaggaaagc      1579
aatctgtctctcatccctttctctcagtggtggg      1611

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Figure 1. Nucleotide and deduced amino acid sequences of a rat cDNA encoding the 5-HT₆ receptor. Both sequences are numbered from the first ATG (Met¹) of the open reading frame. Putative transmembrane regions (TM) are overlined and numbered 1 through 7. (*) represents a consensus glycosylation site. The arrow indicates the position of the intron.

sulfate, 1 x Denhardt's solution, 4 x SSC, 0.1 % sodium pyrophosphate, 100 µg/ml tRNA, 100 µg/ml denaturated salmon sperm DNA) containing 4 x10⁶ dpm of a labeled antisense- or sense-probe. The latters were synthetized by PCR and corresponded to nucleotides 359-597 in Fig.1. After subcloning in pGEM-4Z (Promega), ³²P-labeled antisense- or sense-strand RNA probes were prepared by *in vitro* transcription using a Riboprobe kit (Promega).

RESULTS

Screening of a rat genomic library with a probe encoding putative transmembrane domains (TM1-TM5) of the rat histamine H₂-receptor DNA led to the identification of DNA fragments encoding an open reading frame of 238 amino acids beginning with a consensus ATG initiator codon (GTCCCCATGGTT) (19), a nonsense codon (TAA) at position -240 and an intron sequence (TTGCAGGTACCC) (20) starting after amino acid 238.

The full length nucleotide sequence was obtained by screening a rat striatal cDNA library with a partial length DNA fragment of λG7A. A protein of 436 amino acids with an estimated molecular weight of 46,922 daltons and exhibiting seven clusters of 20-25 hydrophobic residues was deduced (21). A comparison of this sequence with that of various members of the superfamily of G protein-coupled receptors revealed significant homology, particularly with the putative transmembrane domains of some serotonin receptors i.e. 40-45 % with the 5-HT_{dro1} (22), 5-HT_{1C} (23) and 5-HT₂ (24) receptors.

Transient expression of the encoded protein in COS-7 cells transfected with the pSV5-HT₆ expression vector led to cAMP accumulation upon stimulation by serotonin (table 1), whereas no significant accumulation occurred upon addition of 10 µM serotonin to non transfected COS-7 cells (data not shown). In addition, 5-methoxytryptamine (0.01 µM) and 5-carboxamidotryptamine (1 µM) were found to stimulate by 5 fold over basal level cAMP accumulation in transfected COS-7 cells (data not shown).

These data indicate that the cloned protein encodes a novel serotonin receptor (5-HT₆) positively coupled to adenylate cyclase.

Northern blot analysis of various rat tissues revealed two transcripts of 4.1 kb and ~3.2 kb (Fig. 2A). In cerebral tissues, the highest expression was observed in striatum but a signal was also easily detected in the cerebral cortex, hippocampus and hypothalamus. In peripheral tissues, a low expression was detected in the stomach. Northern blot analysis of guinea pig tissues performed with the same probe revealed a single transcript of ~3.8 kb. The strongest signal was found in striatum and olfactory tubercles (Fig. 2B). A moderate expression was also

TABLE 1

STIMULATION OF cAMP ACCUMULATION IN COS-7 CELLS TRANSIENTLY
TRANSFECTED WITH pSV5-HT₆

Drugs	cAMP accumulation	
	fmol/well	% of basal level
None	81 ± 3	100
Forskolin, 10 µM	880 ± 60**	1,086
Serotonin, 0.01 µM	187 ± 36*	231
" 3 µM	643 ± 71**	794

*P < 0.01 ; **P < 0.001 as compared to basal level (Student's t test).

Cells were incubated for 15 min with the drugs at the indicated concentration. Data are representative of two to three independent experiments, conducted in quadruplicate. (means ± S.E.M. ; n = 8-16.)

easily detected in other central regions. Among the peripheral tissues tested, a faint signal was only observed in adrenals.

In situ hybridization with the antisense probe revealed a highly heterogeneous distribution of transcripts in the rat brain, whereas the corresponding sense probe led to a weak

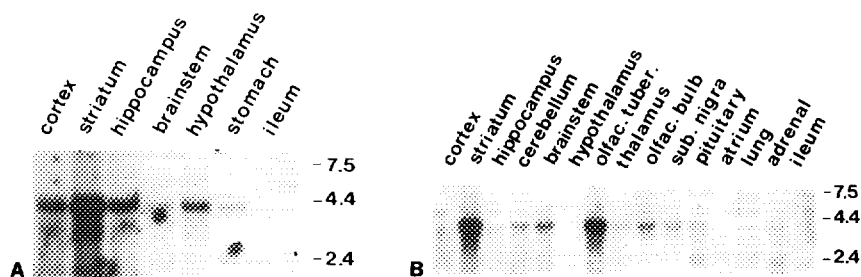


Figure 2. Northern blot analysis of the 5-HT₆ receptor gene transcripts from various rat (A) or guinea pig (B) tissues. Poly(A)⁺ mRNAs (8 µg/lane) were used. Blots were exposed for 4 days at -80°C with intensifying screens. Molecular sizes (kb) are indicated.

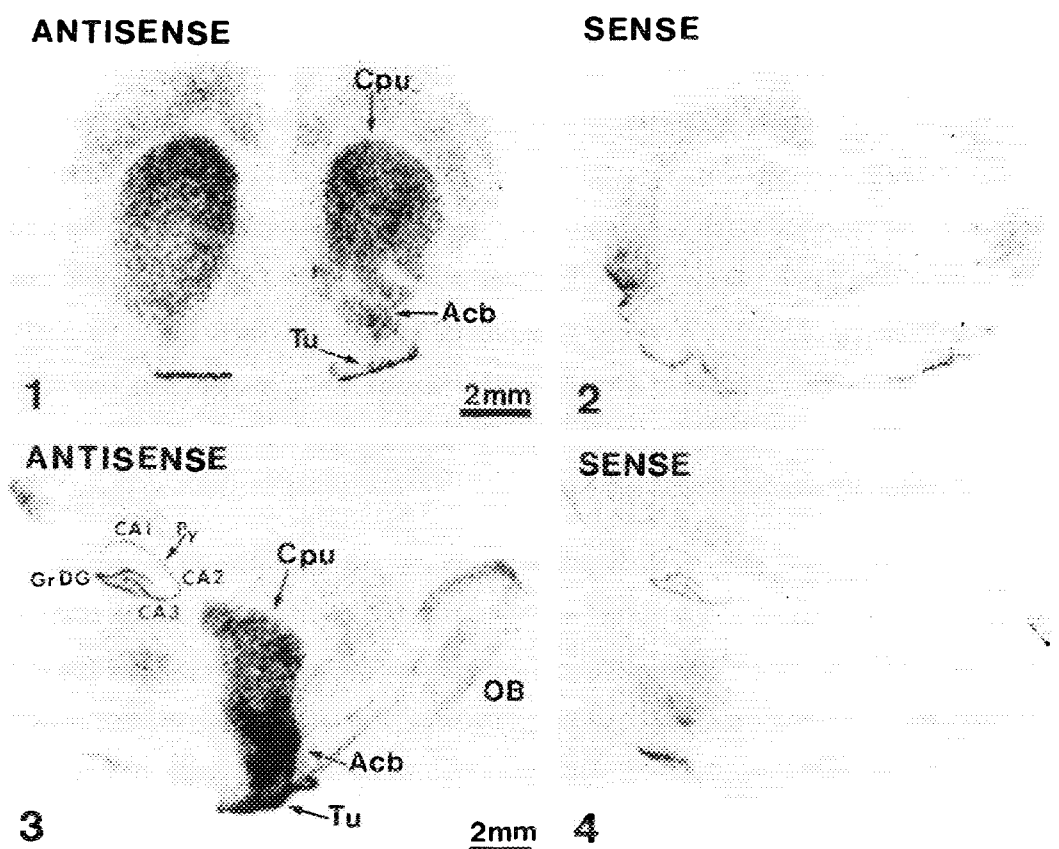


Figure 3. Localization of the 5-HT₆ receptor gene transcripts in frontal and sagittal sections of rat brain by *in situ* hybridization. ³²P-labeled antisense- (1 and 3) or sense-strand (2 and 4) RNA probes were used. Abbreviations : Acb, nucleus accumbens ; CA1-3, fields CA1-3 of Ammon's horn (Py, pyramidal cell layer) ; Cpu, caudate putamen ; DG, dentate gyrus (Gr, granulate layer) ; OB, olfactory bulb ; Tu, olfactory tubercle.

and rather uniform signal (Fig. 3). The highest levels were observed in the olfactory tubercles, striatum and nucleus accumbens. In striatum, the labeling was heterogeneous, possibly indicating a preferential localization in striosomes or matrix. Hybridization signals of moderate intensity also occurred in the hippocampus (CA₁, CA₂ and CA₃ fields and dentate gyrus), olfactory bulb and cerebral cortex.

DISCUSSION

The 5-HT₆ receptor represents the first mammalian serotonin receptor to be cloned that is coupled to the activation of adenylyl cyclase. That this receptor was previously detected in functional studies remains unclear. A positive coupling of the 5-HT_{1A} receptor to adenylyl cyclase in rat brain has been suggested (5, 6). It is clear, however, that the amino acid sequence of the 5-HT₆ receptor differs from that of the cloned 5-HT_{1A} receptor (25). The regional distribution of the 5-HT₆ receptor does not appear to correspond to that of the 5-HT₄ receptor, also positively coupled to adenylyl cyclase (9). The 5-HT₆ receptor may also differ from the 5-HT receptor responsible for a high-affinity activation of adenylyl cyclase in rat cerebral cortex, striatum and hippocampus (7).

The novel 5-HT₆ receptor is characterized by the presence of a consensus glycosylation site located in the amino terminal tail (Asn⁹), of highly conserved cysteine residues (Cys⁹⁹ and Cys¹⁸⁰) presumably involved in a disulfide bond connecting the first and second extracytoplasmic loops and of various consensus sites for phosphorylation by protein kinases C in the third intracytoplasmic loop and in the C terminus. Interestingly, a consensus "leucine zipper" motif (26) is present in the end of the putative third transmembrane domain (Leu¹¹⁵ to Leu¹³⁶) but its structural or functional role remains unclear. An aspartate residue (Asp¹⁰⁶) in TM3, conserved in all aminergic receptors and shown by site-directed mutagenesis (27) to be responsible for salt-linking the ammonium group of amines, presumably plays a similar role in serotonin binding. The structure of this novel serotonin receptor is characterized by a short third cytoplasmic loop (~50 amino acids) and a rather long C-terminal tail (~120 amino acids), two features widely found in receptors positively coupled to adenylyl cyclase (28).

The novel 5-HT₆ receptor displays significant but rather limited homology with other 5-HT receptors, the highest being found with a *Drosophila* serotonin receptor positively coupled to adenylyl cyclase (22). Significant homology also occurs with the rat histamine H₂ receptor, which accounts for its cloning using a probe derived from the sequence of the latter. The 5-HT₆ receptor gene, like those of the 5-HT₂ subfamily (29), i.e. the 5-HT_{1C} and 5-HT₂ itself, has its coding sequence interrupted by at least one intron. The latter occurs at the level of the third intracytoplasmic loop, where an intron is also present in various dopamine receptor genes (30).

The 5-HT₆ receptor gene seems to be expressed in many serotonergic brain areas and in the gastrointestinal tract, as shown by Northern blot analysis. This approach, as well as *in situ* hybridization, indicates that, in the rat, the highest expression level occurs in the basal forebrain (striatal complex), hippocampus, hypothalamus and cerebral cortex. A slightly different distribution seems to occur in guinea pig brain. Species differences in the localization and pharmacology of the same receptor is not uncommon among members of the large 5-HT receptor family (3). Stable expression of the 5-HT₆ receptor in a transfected cell line is required to establish precisely its pharmacology in several species and is in progress in our laboratory.

REFERENCES

1. Julius, D. (1991) *Annu. Rev. Neurosci.* 14, 335-360.
2. Peroutka, S.J. (1993) *J. Neurochem.* 60, 408-416.
3. Zifa, E. and Fillon, G. (1992) *Pharmacol. Rev.* 44, 401-458.
4. Hen, R. (1992) *Trends Pharmacol. Sci.* 13, 160-165.
5. Markstein, R., Hoyer, D. and Engel, G. (1986) *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 333, 335-341.
6. Shenker, A., Maayani, S., Weinstein, H. and Green, J.P. (1987) *Mol. Pharmacol.*, 31, 357-367.
7. Fayolle, C., Fillion, M.P., Barone, P., Oudar, P., Rousselle, J.C. and Fillion, G. (1988) *Fundam. Clin. Pharmacol.* 2, 195-214.
8. De Vivo, M. and Maayani, S. (1990) *Biochem. Pharmacol.* 40, 1551-1558.
9. Bockaert, J., Fozard, J.R., Dumuis, A. and Clarke, D.E. (1992) *Trends Pharmacol. Sci.* 13, 141-145.
10. Quach, T.T., Rose, C., Duchemin, A.M. and Schwartz, J.C. (1982) *Nature* 298, 373-375.
11. Ruat, M., Traiffort, E., Arrang, J.M., Leurs, R. and Schwartz, J.C. (1991) *Biochem. Biophys. Res. Commun.* 179, 1470-1478.
12. Kenakin, T.P., Bond, R.A. and Bonner, T.I. (1992) *Pharmacol. Rev.* 44, 351-362.
13. Plassat, J.L., Boschert, U., Amlaiky, N. and Hen, R. (1992) *EMBO J.* 11, 4779-4786.
14. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463-5467.
15. Saiki, R.K., Gelfand, D.H., Stoffel, S., Sharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. (1988) *Science* 239, 487-491.
16. Sokoloff, P., Giros, B., Martres, M.P., Bouthenet, M.L. and Schwartz, J.C. (1990) *Nature* 347, 146-151.

17. Traiffort, E., Ruat, M., Arrang, J.M., Leurs, R., Piomelli, D. and Schwartz, J.C. (1992) *Proc. Natl. Acad. Sci. USA* 89, 2649-2653.
18. Bouthenet, M.L., Souil, E., Martres, M.P., Sokoloff, P., Giros, B. and Schwartz, J.C. (1991) *Brain Res.* 564, 203-219.
19. Kozak, M. (1987) *Nucl. Acids Res.* 15, 8125-8148.
20. Andreadis, A., Gallego, M.E. and Nadal-Girard, B. (1987) *Ann. Rev. Cell. Biol.* 3, 207-242.
21. Hopp, T.P. and Woods, K.R. (1981) *Proc. Natl. Acad. Sci. USA* 78, 3824-3828.
22. Witz, P., Amlaiky, N., Plassat, J.L., Maroteaux, L., Borelli, E. and Hen, R. (1990) *Proc. Natl. Acad. Sci. USA* 87, 8940-8944.
23. Julius, D., MacDermott, A.B., Axel, R. and Jessell, T.M. (1988) *Science* 241, 558-564.
24. Pritchett, D.B., Bach, A.W., Wozny, M., Taleb, O., Dal Toso, R., Shih, J.C. and Seeburg, P.H. (1988) *EMBO J.* 7, 4135-4140.
25. Kobilka, B.K., Frielle, T., Collins, S., Yang-Peng, T., Kobilka, T.S., Francke, U., Lefkowitz, R.J. and Caron, M.G. (1987) *Nature* 329, 75-79.
26. Landschulz, W.H., Johnson, P.F. and McKnight, S.L. (1988) *Science* 240, 1759-1764.
27. Savarese, T.M. and Fraser, C.M. (1992) *Biochem. J.* 283, 1-19.
28. Lefkowitz, R.J. and Caron, M.G. (1988) *J. Biol. Chem.* 263, 4993-4996.
29. Stam, N.J., Van Huizen, F., Van Alebeek, C., Brands, J., Dijkema, R., Tonnaer, J.A.D.M. and Olijve, W. (1992) *Eur. J. Pharmacol.* 227, 153-162.
30. Schwartz, J.C., Giros, B., Martres, M.P. and Sokoloff, P. (1992) *Sem. Neurosci.* 4, 99-108.