Molecular Cloning and Expression of a 5-Hydroxytryptamine\textsubscript{7} Serotonin Receptor Subtype

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We have utilized the polymerase chain reaction technique to selectively amplify a G protein-coupled receptor cDNA from rat kidney proximal convoluted tubule mRNA, which exhibits high homology with previously cloned serotonin receptors. Sequencing of a full-length clone isolated from a rat hippocampal cDNA library revealed an open reading frame of 1,212 base pairs encoding a 404-residue protein with seven hydrophobic regions predicted to represent transmembrane-spanning domains. Within the transmembrane regions, this receptor was found to be 44-50% identical with various members of the 5-HT\textsubscript{1}, 5-HT\textsubscript{2}, and 5-HT\textsubscript{6} subfamilies with lower (37-40%) homology to the 5-HT\textsubscript{2} like receptors. Northern blots revealed a 3.6-kilobase transcript localized in various brain regions with the following rank order of abundance: hypothalamus > mesencephalon > cerebrospinal fluid > olfactory bulb > olfactory tubercle. Expression of this clone in COS-7 cells resulted in the appearance of high affinity, saturable binding of \textsuperscript{3}H]lysergic acid diethylamide (\textsuperscript{3}H]LSD; \textit{Kd} = 5 nm) and \textsuperscript{3}H]serotonin (\textsuperscript{3}H]5-HT; \textit{Kd} = 1 nm). Among endogenous biogenic amines, only 5-HT completely inhibited radioligand binding. The inhibition of radioligand binding by other serotoninergic agents revealed a pharmacological profile that does not correlate with any previously described serotonin receptor subtype. In addition, this receptor exhibits high affinity for a number of tricyclic antidepressant drugs including clozapine, loxapine, and amitriptyline. In HEK-293 cells stably transfected with this receptor, serotonin elicits a potent stimulation of adenylylcyclase activity. The distinct structural and pharmacological properties of this receptor suggest that it represents a completely novel serotonin receptor subtype, which we propose to designate 5-HT\textsubscript{7}. Based on its pharmacology and its localization to limbic and cortical regions of the brain, it is likely that this receptor may play a role in several neuropsychiatric disorders that involve serotonergic systems.

Serotonin is a ubiquitous neurotransmitter, which is found in both the central and peripheral nervous systems of many species and is involved in a wide variety of behavioral and physiological functions. The transduction of serotonergic signals across cellular membranes is mediated by a diversity of receptor subtypes, which, in mammals, appear to fall into six pharmacologically distinct classes designated 5-hydroxytryptamine\textsubscript{1} (5-HT\textsubscript{1}) through 5-HT\textsubscript{6}. With the exception of the 5-HT\textsubscript{2} receptor, which is a ligand-gated ion channel (1), all of the known serotonin receptor subtypes belong to the large super gene family of G protein-linked receptors. The primary structures for a number of these receptors have been elucidated by molecular cloning, including the 5-HT\textsubscript{1A-F} subtypes (2-15), the 5-HT\textsubscript{2A-C} types (16-21), the 5-HT\textsubscript{5A} & \textsubscript{\alpha} receptors (22, 23), and the 5-HT\textsubscript{6} receptor (24). In addition, three different \textit{Drosophila} serotonin receptors, 5-HT\textsubscript{DRO} (25) and 5-HT\textsubscript{DRO2} & \textsubscript{\alpha} (26), have been cloned and sequenced as well as a serotonin receptor from \textit{Lymnaea stagnalis} (27).

We now report the cloning from rat brain of a cDNA encoding a novel serotonin receptor, which contains seven predicted transmembrane domains but is distinct from any class of previously described 5-HT receptor in both primary structure and pharmacology. This receptor appears to be localized predominantly in the central nervous system, exhibiting high expression in various limbic and cortical brain regions. When expressed in mammalian cells, this receptor shows high affinity for serotonin as well as a number of tricyclic antidepressant drugs including clozapine, loxapine, and amitriptyline. Based on its affinity for these psychotropic drugs and its localization to limbic and cortical regions of the brain, it is likely that this receptor may play a role in several neuropsychiatric disorders which involve serotonergic systems. We propose to designate this novel serotonin receptor the 5-HT\textsubscript{7} subtype.

EXPERIMENTAL PROCEDURES

PCR and Sequencing—Total RNA was prepared from rat kidney proximal convoluted tubules (PCT) using RNAzol B (Cinna/Biotex Laboratories International), and poly(A)+ RNA was isolated using poly(A)Quick columns (Stratagene). First strand cDNA synthesized from the rat PCT mRNA was PCR amplified as described (24) with 1 \muM of each of the following primers: 5'-GTCGACCC(GT)T(GT)(CG)GCC(AC)TCA-(GT)CA/TC(GA)(GA)(TG)(GC)GCTA-3' and 3'-A(GA)(GA)(TA)(GC)ATGCCTAGA-3'. The reaction products were purified by ultrafiltration with Centricon 30 units (Amicon), digested with SaI and HindIII, and isolated on a 1% agarose gel.

\textsuperscript{1} The abbreviations used are: 5-HT, 5-hydroxytryptamine, PCT, proximal convoluted tubule; PCR, polymerase chain reaction; LSD, lysergic acid diethylamide; TM, transmembrane; GppNHp, guanylyl 5'-imidodiphosphate; bp, base pair(s); kb, kilobase(s).

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank\textsuperscript{TM} /EMBL Data Bank with accession number(s) L15228.

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Individual bands were excised, electroeluted, and sequenced. Nucleotide sequence analysis was performed using the Sanger dideoxynucleotide chain termination method with Sequenase (U.S. Biochemical Corp.) on denatured double-stranded plasmid templates. Primers were synthetic oligonucleotides that were vector-specific or derived from prior sequence information. cDNA sequence was confirmed through the sequencing of both strands.

**Northern Blot Analysis**—Poly(A)\(^+\) RNA was prepared from rat tissues and Northern blotting performed as described previously (24). Northern blots were probed with the -500-bp PCR amplification product, which was \(^{32}P\)-labeled by the random primer method, and exposed for 15 days at -70 \(^\circ\)C to x-ray film with an intensifying screen.

**Library Screening**—Recombinants (1 x 10\(^5\)) from a rat hippocampal cDNA library, constructed in the Agt11 vector (Stratagene), were screened with the PCR fragment that was \(^{32}P\)-labeled via nick translation. Library screening and plaque purification was performed as described previously (24). One positive clone containing a 2.9-kb insert was identified, from which a 1.5-kb fragment was excised using EcoRI and subcloned into pBluescript SK II (+). Nucleotide sequence was determined as described above.

**Expression and Radioligand Binding Assays**—For transient expression of the PCT-65 receptor, COS-7 cells were transfected with the pSho-PCT65 construct using the calcium phosphate precipitation method (for \(^{3}H\)-LSD binding assays) as previously described (24) or the DEAE-dextran method (for \(^{3}H\)-HT binding assays) as described by Cullen (28). Stably transfected HEK-293 cells were generated and screened as described in Ref. 24. \(^{3}H\)-LSD (67 Ci/mmol, Du Pont-New England Nuclear) binding experiments were performed as previously described for \(^{125}I\)-labeled L$\sigma$D (24), except that the assay volume was 1 ml containing 30 \(\mu\)g of membrane protein. 5-HT (100 \(\mu\)M) was utilized to determine nonspecific binding, which represented 2–20% of the total binding over the range of concentrations employed in saturation experiments. \(^{3}H\)-HT (25-27 Ci/mmol, Du Pont-New England Nuclear) binding experiments were performed as previously described (24) using a final protein concentration of 5–10 \(\mu\)g/ml. Nonspecific binding was determined in the presence of 10 \(\mu\)M 5-HT or 10 \(\mu\)M methiothepin and represented 2 and 10% of the total binding over the range of concentrations employed. Intracellular CAMP levels in stably transfected HEK-293 cells were determined as described in Ref. 5.

**RESULTS AND DISCUSSION**

Following the original report of Libert et al. (29), we have used the polymerase chain reaction (PCR) in an attempt to clone novel members of the G protein-coupled receptor family. Degenerate oligonucleotide primers were prepared using sequences from the third and sixth transmembrane (TM) regions of previously cloned biogenic amine receptors and used to amplify cDNA synthesized from poly(A)\(^+\) RNA purified from rat kidney proximal convoluted tubules. This resulted in the amplification of a number of cDNA fragments which were determined by DNA sequence analysis to represent portions of previously cloned receptors, some novel receptors as well as unknown DNA sequences (data not shown). One putatively novel receptor PCR fragment, termed PCT-65, exhibited high homology with previously cloned members of the serotonin receptor family and was selected for further study.

In an initial characterization of PCR fragment PCT-65, we examined the tissue distribution of its corresponding mRNA. Fig. 1 shows Northern blots of poly(A)\(^+\) RNA prepared from a variety of rat brain regions as well as other peripheral tissues and probed with the PCT-65 fragment. A single transcript of \(-3.6\) kb was observed in various brain regions with highest expression appearing to occur in the hypothalamus. PCT-65 mRNA was also observed in high abundance in the hippocampus and mesencephalon with lower amounts found in the cortex, olfactory bulb, and olfactory tubercle, whereas no transcript was observable in the cerebellum, pituitary, or retina. In peripheral tissues, this transcript was only seen in the spleen, whereas it was absent, or present in undetectable levels, in the retina, pituitary, testis, stomach, prostate, ovary, skeletal muscle, lung, liver, kidney, and gut. The inability to detect PCT-65 mRNA in kidney via Northern blotting, despite this being the tissue of origin, suggests that PCT-65 must be a relatively minor transcript in the kidney and/or is restricted to a small population of cells.

Based on the Northern blot analysis, we decided to screen a rat hippocampal cDNA library using the PCT-65 PCR fragment in order to obtain a full-length clone. One positive clone was isolated, which contained a cDNA insert of \(-2.9\) kb. A 1.5-kb EcoRI fragment was excised from this insert and subcloned into pBluescript for characterization. The complete nucleotide sequence of the EcoRI fragment was determined and is shown in Fig. 2. This cDNA exhibits a long open reading frame containing sequences that show homology to TM regions of previously cloned biogenic amine receptors. Careful inspection of the sequence also revealed the presence of putative intronic sequence containing an in-frame stop codon. This putative intron is located downstream of the third predicted TM domain of the receptor and suggests that the cDNA was derived from an incompletely or incorrectly spliced mRNA. This possibility was further investigated by using PCR to amplify this region from cDNA prepared from rat hippocampal mRNA. Amplification across this region using primers P1 and P2 (Fig. 2) resulted in two equally abundant cDNA products of approximately 460 and 390 bp in size (data not shown). The larger fragment is the size expected based on the sequence of the isolated cDNA clone, whereas the smaller fragment is the size predicted from the postulated splice sites shown in Fig. 2. Sequencing of the smaller PCR product confirmed the predicted splice sites, which indicates that there are two forms of PCT-65 mRNA in the hippocampus, only one of which is correctly spliced. Splicing at nucleotide 416, as shown in Fig. 2, results in an uninterrupted Arg codon and a 1,213-bp open reading frame encoding a protein of 404 amino acids. It would be noted that without identifying an in-frame stop codon prior to the initiator methionine, the assignment of the NH\(_2\) terminus of this protein is tentative until additional 5' sequence is acquired. The calculated molecular mass of the 404-residue PCT-65 receptor protein is 45 kDa.

Hydropathy analysis (data not shown) of the deduced amino acid sequence of the PCT-65 protein suggests the existence of seven hydrophobic domains, which are predicted to represent putative TM regions. When compared to previously cloned G protein-coupled receptors, the TM regions of PCT-65 exhibit high homology to various serotonin (5-HT) receptors, suggesting that it may be a member of this receptor family. Within the TM regions, the following homologies to PCT-65 were calculated: 5-HT\(_{DRO1}\) (56%), 5-HT\(_{LYN}\) (51%), 5-HT\(_{1A}\) (50%), 5-HT\(_{1F}\) (24%).

![Fig. 1. Northern blot analysis of PCT-65 mRNA in rat brain and peripheral tissues.](image)
The Rat 5-HT7 Serotonin Receptor

FIG. 2. Nucleotide and deduced amino acid sequences of cDNA clone PCT-65. The nucleotide sequence is shown in uppercase letters, noncoding sequence is shown in lowercase letters, and deduced amino acid sequence is shown below the nucleotide sequence. Sequences of the PCR primers described in the text are boxed. Putative TM domains are indicated by lines above the sequences. Solid inverted triangles indicate the positions of the BclI restriction sites used in the construction of an intronless version of this clone. An Asn residue representing a putative glycosylation site is underlined.

(48%), 5-HT1D (47%), 5-HT5A & B (46%), 5-HT1A (45%), 5-HT1E (45%), 5-HT1F (44%), 5-HT1D/B (44%), 5-HT1D/B (43%), and 5-HT1B (39%), and among 37% of the 5-HT7 or 5-HT3) Thus, among the previously cloned 5-HT receptors, PCT-65 is most homologous with the Drosophila 5-HTD/B and L. stagnalis 5-HTV receptors. Among the mammalian receptors, PCT-65 exhibits TM homologies of 44–50% to the 5-HT1, 5-HT2, and 5-HT3 receptor subfamilies but has lower (37–40%) homology to the 5-HT2 subfamily.

The predicted third cytoplasmic loop of the PCT-65 receptor is relatively short, consisting of about 60 residues, whereas the approximately 65-residue-long carboxyl-terminal tail is intermediate in length in comparison to other cloned 5-HT receptors. The PCT-65 receptor protein also contains one potential N-linked glycosylation site at Asn-25 in the extracellular amino terminus, in addition to several potential sites for phosphorylation by cAMP-dependent protein kinase or protein kinase C in the cytoplasmic loops and carboxyl terminus.

In order to definitively establish the identity of the receptor encoded by PCT-65, we proceeded with its expression in mammalian cells. As the intron-containing clone proved unsuitable for expression, we generated an appropriately spliced cDNA. We first utilized the correctly spliced cDNA fragment, generated using PCR primers P1 and P2 as described above, and digested it with BclI, the sites of which are indicated by solid triangles in Fig. 2. The resulting BclI fragment was subsequently ligated into the full-length BclI-digested cDNA clone. We were thus able to excise the intron-containing BclI fragment from the cDNA isolated from the hippocampal library and replace it with the fragment corresponding to a PCR-amplified correctly spliced cDNA. This construct was subcloned into the mammalian expression vector pCD-SRa (30) yielding pSRo-PCT65.

Transient transfection of COS-7 cells with pSRo-PCT65 resulted in the appearance of high affinity, homogeneous, and saturable binding sites for the serotonergic ligand [3H]LSD, which exhibited a $K_d$ of 4.9 ± 0.78 nM and $B_{max}$ values ranging from 5–15 pmol/mg protein (data not shown). No specific binding of [3H]LSD was observed in untransfected COS-7 cells or in cells transfected with the pCD-SRa vector alone. Preliminary characterization of the PCT-65 pharmacology indicated that among several endogenous biogenic amines, including dopamine, melatonin, epinephrine, norepinephrine, or histamine, 5-HT was the most potent in competing for [3H]LSD binding sites, exhibiting a $K_d$ of 1.2–2 nm (Table I). The Hill coefficient for the 5-HT competition curve was not significantly different from unity, and adding the guanine nucleotide analogue GppNHp (0.3 mM) to the assay had no effect (data not shown).

The binding of 5-HT to PCT-65 was also investigated directly using [3H]5-HT as the radioligand. Saturation analysis resulted in the appearance of high affinity, homogeneous, and saturable binding sites for the serotonergic ligand [3H]LSD, which exhibited a $K_d$ of 4.9 ± 0.78 nM and $B_{max}$ values ranging from 5–15 pmol/mg protein (data not shown). No specific binding of [3H]LSD was observed in untransfected COS-7 cells or in cells transfected with the pCD-SRa vector alone. Preliminary characterization of the PCT-65 pharmacology indicated that among several endogenous biogenic amines, including dopamine, melatonin, epinephrine, norepinephrine, or histamine, 5-HT was the most potent in competing for [3H]LSD binding sites, exhibiting a $K_d$ of 1.2–2 nm (Table I). The Hill coefficient for the 5-HT competition curve was not significantly different from unity, and adding the guanine nucleotide analogue GppNHp (0.3 mM) to the assay had no effect (data not shown).

The following compounds, which are not listed in the table, were tested and found to exhibit high affinity, saturable binding sites in transfected, but not untransfected, COS-7 cells demonstrating a $K_d$ of 0.97 ± 0.1 nm (n = 5) and $B_{max}$ values of 2–10 pmol/mg protein (data not shown).

**Table I**

| Drug                                      | $K_d$ (mean ± S.E.) | $B_{max}$ (mean ± S.E.) |
|-------------------------------------------|---------------------|--------------------------|
| 5-Carboximidotryptamine                   | 0.33 ± 0.01         | 0.16 ± 0.05              |
| Lisuride                                   | 0.52 ± 0.12         | 0.89 ± 0.32              |
| Methiothepin                               | 1.03 ± 0.16         | 0.38 ± 0.18              |
| 5-Methoxytryptamine                       | 1.78 ± 0.05         | 0.57 ± 0.03              |
| 5-Hydroxytryptamine                       | 1.83 ± 0.07         | 1.52 ± 0.27              |
| Metergoline                                | 2.94 ± 0.15         | 6.21 ± 1                 |
| Perpudol                                   | 4.78 ± 0.18         | 10.1 ± 0.03              |
| Meulergine                                 | 7.13 ± 0.39         | 21.2 ± 2.19              |
| Metergoline                                | 10.7 ± 0.47         | 45.8 ± 7.9               |
| Methysergide                               | 12.5 ± 0.84         | 13.4 ± 0.8               |
| Clozapine                                  | 13.5 ± 2.51         | 39.5 ± 9.6               |
| 5-Methoxy-N,N-dimethyltryptamine           | 20.9 ± 0.95         | 8.20 ± 0.73              |
| Ritonatine                                 | 21.7 ± 2.1          | 15.3 ± 3.4               |
| Tryptamine                                 | 32.8 ± 0.76         | 16.4 ± 1.01              |
| 1(1-Naphthy)piperazine                     | 33.7 ± 1.68         | 18.3 ± 0.77              |
| 8-OH-DBAT                                  | 35.4 ± 5.24         | 35 ± 0.62                |
| Cyproheptadine                             | 36.5 ± 4.26         | 31 ± 4.15                |
| Mianserin                                  | 111 ± 13.8          | 30.9 ± 2.6               |
| Amtriptiline                               | 123 ± 13.3          | 92.5 ± 15.8              |
| Loxapine                                   | 171 ± 7.8           | 133 ± 3.11               |
| Ketanserin                                 | 206 ± 3.5           | 265 ± 9.5                |
| 2-MPP                                      | 243 ± 16.4          | 125 ± 8.4                |
| mCPP                                       | 352 ± 54.9          | 256 ± 9.5                |
| TFMP                                       | 532 ± 33            | 236 ± 5.3                |
| Sumatriptan                                | 570 ± 49            | 291 ± 36.1               |
TABLE II

| Drug treatment | cAMP pmol/well |
|---------------|--------------|
| Basal         | 47 ± 9       |
| Forskolin (100 µM) | 900 ± 41     |
| 5-HT (100 nM) | 310 ± 58     |
| Methiothepin (5 µM) | 68 ± 18      |
| Clozapine (10 µM) | 75 ± 16      |
| 5-HT (100 nM) + methiothepin (5 µM) | 74 ± 17     |
| 5-HT (100 nM) + clozapine (10 µM) | 76 ± 16     |

Shown). Inclusion of 0.3 mM GTP did not appreciably alter the binding of [3H]5-HT. These initial radioligand binding assays suggest that PCT-65 represents a 5-HT receptor subtype. The apparent absence of receptor-G protein coupling in the transiently transfected COS cells, as evidenced by the lack of guanine nucleotide regulation of agonist binding, may be due to either an excess of receptor expression relative to the endogenous G proteins, or, alternatively, the cells may not express the appropriate G protein(s) that is normally linked to the PCT-65 receptor.

Further characterization of the PCT-65 pharmacology involved competition assays using a variety of drugs that exhibit specificity for various serotonergic receptor subtypes and other binding sites. The average $K_i$ values for compounds competing with $100$ nM serotonin are shown in Table I. Examination of the rank order of potency for a variety of serotonergic agents reveals that the pharmacology of clone PCT-65 does not correspond to any previously described serotonin receptor subtype. A number of drugs that exhibit high affinity for the 5-HT$_1$ receptor (1,13-chlorophenyl)piperazinyl and 1,2-methoxyphenyl)piperazine, 5-HT$_2$ (ketanserin and mianserin), 5-HT$_3$ (quipazine and MDL 72225), and 5-HT$_4$ (zacopride and DAU 6285) receptor subfamilies exhibit relatively low affinity for PCT-65. Ergot alkaloids, especially ergoline derivatives (i.e. LSD, lisuride, or metergoline), display relatively high affinity for PCT-65 as does the non-selective serotonin antagonist methiothepin. The 5-HT$_7$ receptors similarly exhibit high affinity for ergot alkaloids but differ from PCT-65 in having low affinity for 5-HT$_7$ and methiothepin (22, 23). Interestingly, the atypical and typical anti-psychotics clozapine and loxapine, respectively, also exhibit high affinity for PCT-65, as does the tricyclic anti-depressant amitriptyline. In general, the drugs that exhibit the greatest affinity for PCT-65 (i.e. $K_i < 500$ nM) are tricyclic, ergoline, or tryptamine derivatives. This is reminiscent of the recently described 5-HT$_{6}$ receptor (24), which exhibits a similar but distinctive pharmacology.

To investigate possible effects on second messenger levels, we stably transfected HEK-293 cells with the pSRa-PCT-65 construct. One cell line, expressing about 800 fmol of [3H]5-HT binding activity/mg of protein, was selected for further analysis. Serotonin was found to elicit a dose-dependent stimulation of cAMP levels in these cells exhibiting an EC$_{50}$ of 63 ± 4 nM (n = 2), whereas there was no response in nontransfected cells (data not shown). Maximum cAMP accumulation was observed using 10 µM serotonin, which produced a 10–15-fold stimulation over basal levels. The serotonin response was pharmacologically specific being blocked by methiothepin and clozapine (Table II). These data indicate that the PCT-65 receptor is linked to activation of the adenylcyclase signal transduction system.

Taken together, our data indicate that we have identified and cloned a prototypical member of a seventh subtype of 5-HT receptors. This receptor is structurally (<500 homology in the TM domains) and pharmacologically distinct from any previously cloned mammalian 5-HT receptor. On this basis, we propose to designate clone PCT-65 the 5-HT$_7$ subtype. The 5-HT$_7$ receptor does, however, exhibit significant structural and pharmacological similarities to two previously cloned non-mammalian receptors, the 5-HT$_{DRO1}$ (25) and 5-HT$_{LYM}$ (27), suggesting that it may represent a mammalian homolog of one of these receptor subtypes.

Another feature that distinguishes the 5-HT$_7$ receptor from many previously cloned serotonin receptors is the presence of at least one intron in the coding region of its gene. Thus far, all of the 5-HT$_1$ receptor subtypes appear to be encoded by intron-less genes (13), whereas we estimate that at least two introns in the gene (33, 34). The 5-HT$_{5A}$ and 5-HT$_{5B}$ receptors each have a single intron interrupting their coding sequences (23), and the 5-HT$_{5C}$ receptor also has at least one intron in its gene (24). Interestingly, the position of the intron in the 5-HT$_7$ receptor gene (within the codon for Arg-139) does not correlate with the position of any of the introns within the genes encoding the 5-HT$_{5A}$ and 5-HT$_{5C}$ receptor subfamilies nor with the only known intron in the 5-HT$_{6}$ receptor gene. There is, however, an intron at the identical position within the genes encoding the D$_2$ (35), D$_3$ (36), and D$_4$ (37) dopamine receptors (the D$_2$ subfamily). This might suggest that the 5-HT$_7$ receptor is evolutionarily related to the D$_2$ dopamine receptors. Interestingly, the 5-HT$_7$ receptor is fairly homologous to D$_2$-like receptors exhibiting identities of 46% (D$_2$), 48% (D$_3$), and 42% (D$_4$) within the TM regions.

Another interesting feature concerning the intron identified in the 5-HT$_7$ receptor cDNA is that it appears to exhibit a non-consensus 5` splice site. In our initial characterization of the intron-containing cDNA (Fig. 2), we attempted to express it in COS-7 cells. Using PCR analysis, however, we found that cDNA transcribed from this clone did not undergo further splicing, nor did it impart receptor binding activity to the cells (data not shown). This suggests that the 5` splice site in the cDNA clone is not capable of functional splicing, at least in the COS-7 cells. We are currently postulating that there is actually additional 5` sequence in this intron region of the gene and that this cDNA was derived through alternative splicing at an internal acceptor site producing a non-functional mRNA. Testing of this hypothesis will require the isolation and characterization of the 5-HT$_7$ receptor gene. Confirmation of the PCR-generated, correctly spliced cDNA has recently been obtained from isolating and sequencing additional 5-HT$_7$ receptor clones from both rat and human cDNA libraries (data not shown).

Although the pharmacology of the 5-HT$_7$ receptor is distinct and does not correspond to any previously characterized subtype, it does exhibit high affinity for a number of ligands that interact with the 5-HT$_7$ receptor family. For instance, its high affinity for 5-HT and 5-carboxamidotryptamine suggest that it may have been included in previous characterizations of "multiple" 5-HT receptor binding sites using [3H]5-HT or [3H]5-carboxamidotryptamine as radioligands (38). Similarly, the 5-HT$_7$ receptor exhibits relatively high affinity for the classic 5-HT$_{1A}$ receptor agonist, 8-hydroxy-N,N-dipropyl-2-aminoetralin, suggesting that it might have contributed to previously described "5-HT$_{1A}$" responses. Perhaps of greater interest is the demonstrated high affinity of the 5-HT$_7$ receptor for a number...
of therapeutically important psychotropic drugs including the atypical antipsychotic, clozapine. Clozapine is unique from other clinically used antipsychotic drugs due to its activity in patients resistant to other medications and by its lower propensity to cause extrapyramidal side effects. At ~15 nM, the affinity of the 5-HT7 receptor for clozapine is similar to that of the D4 dopamine, 5-HT3 subfamily, and 5-HT6 serotonin receptors and further suggests that it is occupied at therapeutically employed concentrations. Given this consideration in addition to its demonstrated localization to limbic and cortical regions of the brain, it seems likely that the 5-HT7 receptor may play an important role in the therapy of psychotic disorders. The identification and cloning of this novel receptor subtype should enable testing of this hypothesis as well as further investigations of its role in normal physiology and behavior.

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