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Cloning, expression and pharmacology of a truncated splice variant of the human 5-HT_7 receptor (h5-$HT_{7(b)}$)

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1 The rat 5-hydroxytryptamine (5-HT)$_7$ receptor displays two splice variations, a long form, and a truncated splice isoform, arising from the introduction of a stop codon near the carboxy-terminus. The human 5-HT_7 receptor gene contains at least two introns and encodes a 445 amino acid 5-HT receptor.

2 A truncated splice variation in the human 5-HT_7 receptor was isolated from a human placental cDNA library. In accordance with current NC-IUPHAR nomenclature guidelines, it is suggested that this receptor be denoted as the h5-$HT_{7(b)}$ receptor and the long form of the receptor as h5-$HT_{7(a)}$.

3 The h5-$HT_{7(b)}$ receptor was stably expressed in HEK 293 cells and ligand affinities were determined by displacement of [^{3}H]-5-carboxyamidotryptamine (5-CT; $K_d = 0.28 \pm 0.06$ nM, $B_{max} = 7.3 \pm 1.7$ pmol mg^{-1} protein). The rank order of affinities (pK_i) for a series of ligands was: 5-carboxamidotryptamine (5-CT, 9.65) > 5-hydroxytryptamine (5-HT, 9.41) > methiothepin (8.87) > mesulergine (7.87) > 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT, 6.85) > ketanserin (6.44).

4 The h5-$HT_{7(b)}$ receptor coupled positively to adenylyl cyclase in HEK 293 cells. This response was elicited by a number of agonists with the following order of potency (pEC_{50}): 5-CT (8.7 ± 0.11) > 5-MeOT (5-methoxytryptamine; 8.1 ± 0.20) > 5-HT (7.5 ± 0.13) > tryptamine (5.6 ± 0.36) > 8-OH-DPAT (5.3 ± 0.28) > 5-methoxytryptamine (5.0 ± 0.06). This rank order was comparable to that observed in the radioligand binding studies.

5 In a similar fashion to that described for the 5-$HT_{7(a)}$ receptor, PCR studies suggested that the 5-$HT_{7(b)}$ receptor mRNA is found in great abundance throughout the brain, in the small intestine and aorta.

6 It is concluded that the h5-HT_7 receptor, like the rat receptor, exists as splice variants exhibiting similar pharmacology, signal transduction and distribution. It is thus likely that there exists a complex physiological role for alternate splicing products of the 5-HT_7 receptor gene.

Keywords: 5-HT_7 receptor; 5-hydroxytryptamine (5-HT, serotonin); orphan receptor; splice variant

Introduction

The pharmacology of both recombinant and endogenous 5-HT_7 receptors corresponds to several 'orphan' 5-HT receptors (see Eglen *et al*., 1997 for review; Bradley *et al*., 1986; Martin, 1994; Schoeffter *et al*., 1996), expressed within the brain and periphery. These include a 5-HT_{1A}-like receptor in guinea-pig hippocampus (Shenker *et al*., 1987) and 5-HT receptors mediating relaxation of porcine vena cava (Sumner *et al*., 1989), canine coronary artery (Terron, 1996), guinea-pig ileum (Carter *et al*., 1995) and *Cynomologus* monkey jugular vein (Leung *et al*., 1996). Furthermore, the 5-HT_7 receptor probably mediates the tertiary, prolonged hypotensive response to i.v. injection of 5-HT in the rat (Kalkman *et al*., 1983; Martin *et al*., 1987).

5-HT receptors are defined with operational, structural and transductional data (Hoyer *et al*., 1994; Humphrey *et al*., 1993), and form discrete families, including three subtypes positively coupled to adenylyl cyclase (5-HT_4, 5-HT_6 and 5-HT_7). It is now recognized that alternate mRNA splicing of several 5-HT receptor genes, including the 5-HT_2, 5-HT_3 and 5-HT_4 receptor, adds to this complexity. This phenomenon also appears to be the case for the human and rat 5-HT_7 receptor. The h5-HT_7 receptor gene encodes a G-protein coupled receptor with a predicted amino acid sequence of 445 amino acids (Bard *et al*., 1993). The gene contains two introns in the coding region, one located after the third transmembrane domain and the other near the carboxy terminus (Heidmann *et al*., 1997). In the rat, a 5-base pair insertion (GTAAG) at the carboxy region intron introduces an in-frame stop codon, resulting in a thirteen amino acid truncated splice variant (Ruat *et al*., 1993 ; Lovenberg *et al*., 1993).

In the present study, we describe the cloning, expression and characterization of a truncated splice variant, obtained from a human placental cDNA library. This represents a human ortholog of the truncated region found in the rat. Preliminary accounts of these data have been presented previously (Jasper *et al*., 1997).

Methods

Cloning of 5-HT_7 receptor isoforms

h5-$HT_{7(b)}$ receptor Polymerase chain reaction (PCR) primers for the h5-HT_7 receptor were designed from the sequence described by Bard *et al*. (1993; Gen Bank accession number L2 1195). EcoRI restriction tails were incorporated into the primers to facilitate subcloning directly into pSW104m mammalian cell expression vector. 5′ primer: 5′-AGAATTCGGCGG-CGCGATGATGGACGTTAACAGC-3′; 3′ primer: 5′-CGAAT TCCTCTCCAT TGTTCTGCTTTCAATCATGAA-TC-3′. The human placental cDNA template used for PCR amplification was obtained from Clontech (Quickclone cDNA). The PCR conditions used were: 55°C, annealing temperature, 5% formamide, TaKaRa EX-taq DNA polymerase, 35 cycles.

h5-$HT_{7(a)}$ receptor The previously described h5-HT_7 (Bard *et al*., 1993) was derived synthetically by two 79mer oligonucleotides with a 25 bp overlap: 5′-GCATGCATGAAGCCC-TGAAGCT TGCTGAGAG GCCAG AGAGACCTGAGTT-TGTGCTACAAAATGCTGACTACTGTAGAAA-3′ and 5′-TTGCTAGCAATCATGAATCATGACC TTTTTT TCTAC-AGTAGTCAGCAT TTTGTAGCACAAAC TCAGGTCTC-

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TCTGGCCT-3′. An NsiI site was included at the 5′ end and an NheI site was included in the 3′ end to facilitate subcloning. The two oligonucleotides were annealed, filled in and the resulting fragment was amplified by PCR. To reproduce the long variant, a four way ligation was performed by use of the following digested fragments: RI-NheI (pSW104m vector), RI-NsI and NsiI-NsiI (splice variant 5-HT$_7$ clone) and NsiI-NheI (synthetically derived).

After the two oligos had been annealed and filled in, the fragment was PCR amplified. To create the published sequence, a four way ligation was performed with the following digested fragments: RI-NheI (pSW104m vector), RI-NsiI and NsiI-NsiI (splice variant 5-HT$_7$ clone) and NsiI-NheI (synthetically derived published form; Bard *et al*., 1993).

Tissue distribution of human splice variants

PCR was performed against 1 ng Clontech Quickclone cDNAs by use of Touchdown PCR (72°C annealing, 5 cycles; 70°C annealing, 5 cycles; 68°C annealing, 25 cycles); one fifth of the total reaction was loaded on a 1% agarose gel and the products were visualized by ethidium bromide staining.

Cell culture and membrane preparation

Truncated h5-HT$_7$ receptors (clone #58), expressed in HEK-293 cells, were grown in Dulbecco's modified Eagle's medium (DMEM) without sodium pyruvate and with 4.5 gl^{-1} glucose; Gibco) containing 10% foetal bovine serum and 250 μg ml^{-1} G-418 (Geneticin) in a 95% CO_2, 5% O_2 environment at 37°C. Cells (1 million cells ml^{-1}) were homogenized in 50 mM Tris buffer containing 0.5 mM sodium EDTA, 10 mM magnesium sulphate, 2 mM $CaCl_2$, 0.1% ascorbate and 10 μM pargyline, pH = 7.4 at 4°C. The cell homogenate was centrifuged at $45000 \times g$ for 15 min and resuspended twice in the above buffer. The membranes were finally resuspended in the above Tris buffer for binding studies.

Radioligand binding studies

For saturation studies, membranes were incubated for two hours at room temperature with 0.05 – 10 mM [^{3}H]-5-carboxamidotryptamine ([^{3}H]-5-CT; 51.3 Cimmol^{-1}, New England Nuclear, Boston) in the above buffer. To each assay tube were added 200 μl cell membrane, 100 μl radioligand, 150 μl buffer and 50 μl of buffer (total binding) or 50 μl of 1 μM 5-HT, to define non-specific binding. For competition studies, membranes were incubated with 0.3 – 0.7 nM [^{3}H]-5-CT for two hours with or without competing ligands. To each assay tube, 200 μl cell membrane, 100 μl radioligand, 150 μl buffer and 50 μl of buffer (for total) or 50 μl of 1 μM 5-HT (for NSB) or 50 μl of displacing drugs were added. Assays were terminated by rapid filtration through 0.3% polyethylenimine pretreated GF/B filters, washed twice with 1 ml ice cold 50 mM Tris-base, pH = 7.4, and bound radioactivity determined by liquid scintillation spectophotometry with a Packard Topcount scintillation counter.

Cyclic AMP accumulation studies

Preparation of cells Clone #58 cells expressing the h5-HT$_{7(b)}$ receptor were grown in DMEM (without sodium pyruvate, 4.5 gl^{-1} glucose; Gibco) containing 10% foetal bovine serum and 250 μg ml^{-1} G-418 (Geneticin) in a 95% CO_2, 5% O_2 environment at 37°C. The cells were harvested by detaching with 2 mM EDTA/PBS for 2 – 5 min at 37°C and then centrifuged at 2000 r.p.m. for 5 min. Cells were washed once with DMEM with glucose, L-glutamine (without sodium bicarbonate and sodium pyruvate; no phenol red; Gibco) with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES). The cells were adjusted to 2.5×10^6 cells ml^{-1} with DMEM-HEPES containing 2 mM isobutylmethylxanthine (IBMX, to inhibit phosphodiesterase activity; Sigma).

Cyclic AMP assay From the above cell suspension, 200 μl cells (0.5×10^6 cells) were added to either buffer or drug (in duplicate) in a final volume of 300 μl. The tubes were vortexed and incubated at 37°C for 20 min. Cells were pretreated with antagonists (or buffer) for 10 min before the addition of 0.3 μM 5-HT. The reaction was terminated by the addition of 50 μl ice cold perchloric acid (20%). Samples were allowed to sit on ice for 20 min, followed by addition of 80 μl KOH (2 N) in 25 mM HEPES. After a 30 min incubation at room temperature (to allow precipitate to settle), 25 μl of sample were diluted 1 : 3 before addition to a Packard OptiPlate for adenosine 3′:5′-cyclic monophosphate (cyclic AMP) measurement by Scintillation proximity assay (SPA; Amersham).

Analysis of data

Radioligand binding studies The concentration displacing 50% of specific [^{3}H]-5-CT binding (IC_{50}) and the Hill slope, were determined by iterative curve fitting techniques (Graphpad Software, San Diego). The inhibition dissociation constant (K_i) of each compound was then determined, according to the method of Cheng & Prusoff (1973). In general, the negative logarithm of the K_i (pK_i) values are presented. The results presented are the average (±s.e.mean) of at least three independent experiments, unless indicated.

Cyclic AMP accumulation studies The concentration producing 50% of the maximal stimulation of intracellular cyclic AMP (EC_{50}) and Hill slope were determined by iterative curve fitting techniques (GraphPad Software, San Diego). The inhibition dissociation constant (K_i) of each antagonist was determined by a method derived from that of Cheng & Prusoff (1973), with an EC_{50} value of 5-HT in these assays. Typically, the mean (±s.e.mean) negative logarithm of the K_i (pK_i) or EC_{50} (pEC_{50}) for antagonist or agonists, respectively, are presented.

Compounds used

5-Carboxamidotryptamine (5-CT), clozapine, cyproheptadine hydrochloride, ketanserin tartrate, mesulergine hydrochloride, haloperidol, metergoline, methiothepin mesylate, 5-methoxytryptamine (5-MeOT), methysergide maleate, pirenperone, 5-hydroxytryptamine (5-HT) hydrochloride, spiperone hydrochloride and 8-hydroxydipropylaminotetralin hydrobromide (8-OH-DPAT) were purchased from Research Biochemicals International (Natick, MA). 6-Methoxytryptamine (6-MeOT) and tryptamine hydrochloride were obtained from Sigma Chemical Co. Sumatriptan hemisuccinate was synthesized at Roche Bioscience (Palo Alto, CA). [^{3}H]-5-CT (50.4 Cimmol^{-1}) was purchased from Dupont/New England Nuclear (Boston, MA).

Results

Cloning of 5-HT$_7$ receptor splice variants

By use of PCR primers derived from the sequence of the h5-HT$_{7(a)}$ receptor (Bard *et al*., 1993), a novel splice variation from a human placental cDNA library was derived (Figure 1a). The sequence was similar to the coding sequence for the human 5-HT$_7$ receptor, except that the receptor sequence contained a 5 base-pair insertion (GTAAG) near the carboxy-terminal end of the gene, resulting from the use of an alternative splice donor at the exon 2-intron 2 splice junction. This DNA insertion introduces a stop codon (TAA) and terminates the open reading frame and truncates the final thirteen amino acids of the receptor (Figure 1b).

To be consistent with NC-IUPHAR nomenclature recommendations and that proposed by Heidmann *et al*. (1997), we use the term 5-HT$_{7(a)}$ receptor to describe the receptor variant (long form) initially described by Bard and colleagues (1993).

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          1284       1294       1304       1314       1324       1334       1344
h 5-HT7(a) CCTGAGTTTG TGCT.....A CAAAATGCTG ACTACTGTAG AAAAAAAGGT CATGATTCAT GA
          P  E  F  V   L        Q  N  A  D   Y  C  R   K  K  G   H  D  S  *
h 5-HT7(b) CCTGAGTTTG TGCTGTAAGA CAAAATGCTG ACTACTGTAG AAAAAAAGGT CATGATTCAT GA
          P  E  F  V   L  *
          1293       1303       1313       1323       1333       1343       1353
r 5-HT7(a) TCCGAGTTTG TGCT.....A CAAAACTCTG ACCACTGTGG GAAAAAGGGT CATGATACAT GA
          S  E  F  V   L        Q  N  S  D   H  C  G   K  K  G   H  D  T  *
r 5-HT7(b)  TCCGAGTTTG TGCTGTAAGA CAAAACTCTG ACCACTGTGG GAAAAAGGGT CATGATACAT GA
          S  E  F  V   L  *
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Figure 1 Comparison of human and rat 5-HT$_7$ receptor carboxy-terminal nucleotide and amino acid sequences. The sequence data indicate a 5 bp insertion in the human placental cDNA-derived 5-HT$_{7(b)}$ receptor. The insertion introduces an 'in frame' stop codon, shortening the carboxy tail by 13 amino acids (compared to the 5-HT$_{7(a)}$ receptor).

The truncated variant described in the present study is thus to be designated as 5-HT$_{7(b)}$ receptor, by its order of discovery. Since these are sequences of human origin, they are correctly referred to as h5-HT$_{7(a)}$ (long form) and h5-HT$_{7(b)}$ (short form). To aid clarity, this terminology will be used in the remainder of the paper.

Pharmacology of h5-HT$_{7(a)}$ and h5-HT$_{7(b)}$ receptors

Cyclic AMP accumulation studies The pharmacology of the h5-HT$_7$ receptor isoforms was investigated by use of stably transfected HEK 293 cells with both the 5-HT$_{7(a)}$ and 5-HT$_{7(b)}$ receptors. With the 5-HT$_{7(b)}$ clone that expressed the highest receptor density (#58), agonist-mediated cyclic AMP accumulation in whole cells was observed for a number of compounds (Figure 2). The rank order of agonist potency (pEC$_{50}$) at the 5-HT$_{7(b)}$ receptor was: 5-CT (8.7 ± 0.11) > 5-methoxytryptamine (8.1 ± 0.20) > 5-HT (7.5 ± 0.13) > tryptamine (5.6 ± 0.36) > 8-OH-DPAT (5.3 ± 0.28) > 6-methoxytryptamine (5.0 ± 0.06). The accumulation of cyclic AMP, enhanced by 1 μM 5-HT, was inhibited by several antagonists, including methiothepin, metergoline, clozapine, and methysergide (Figure 3; Table 1).

Radioligand binding studies The h5-HT$_{7(b)}$ receptor bound [^{3}H]-5-CT and [^{3}H]-5-HT with high affinity. Binding isotherms with the radioligand [^{3}H]-5-CT yielded a p$K_d = 9.55 \pm 0.10$, $B_{max} = 7.3 \pm 1.7$ pmol mg^{-1} protein (Figure 4). Specific [^{3}H]-5-CT binding was displaced by several ligands, 5-CT being the most potent (Figure 5). Both radioligand binding and agonist-mediated cyclic AMP accumulation in whole cells were compared in the highest expressing clone (#58; Table 1). To investigate potential effects of receptor density on ligand affinity, three clones were studied (Table 2). Clone #58 expressed a median level of 5-HT$_{7(b)}$ receptor density and gave, in general, the highest ligand affinity estimates. Collectively, ligand affinity did not correlate with receptor expression level in clones expressing 2.5–7.3 pmol receptor mg^{-1} protein. In competition radioligand binding studies the agonists 5-CT and 5-HT, and antagonists such as metergoline, displayed high affinity (p$K_i > 8$) for the 5-HT$_{7(b)}$ receptor (Table 1). It was observed that agonist potency or antagonist affinity in the cyclic AMP accumulation studies was less than those affinities measured in the radioligand binding studies (Table 1). The affinity of ligands at both the short (5-HT$_{7(b)}$) and long (5-HT$_{7(a)}$) forms of the receptors were similar (Table 3). However, when these data were compared to ligand affinities, with [^{3}H]-5-HT at the h5-HT$_{7(a)}$ receptor (Bard *et al.*, 1993) minor differences were noted (Table 3), including the affinity of 5-HT (p$K_i = 9.7$, present study; p$K_i = 8.1$, Bard *et al.*, 1993). However, overall the data generated in the present study for both human splice variants compared closely with those values obtained for rat (Lovenberg *et al.*, 1993; Shen *et al.*, 1993; Ruat *et al.*, 1993) and guinea-pig (To *et al.*, 1995) 5-HT$_7$ receptors (Table 3) and mouse 5-HT$_7$ receptors (Plassat *et al.*, 1993).

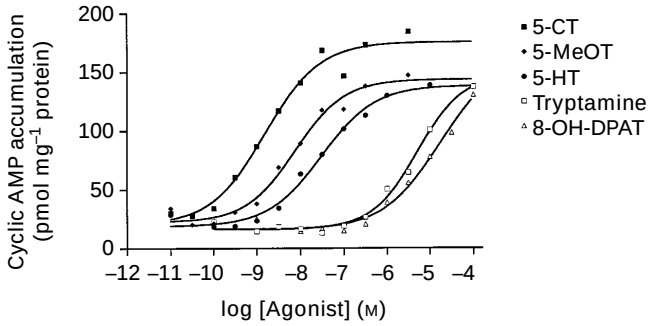

Figure 2 Agonist-mediated cyclic AMP accumulation in 293 cells expressing the human 5-HT$_{7(b)}$ receptor. Whole cells were stimulated for 20 min with agonists in the presence of phosphodiesterase inhibitors. Shown is a single experiment; each curve is representative of at least three individual experiments. The Hill slopes for the concentration-response curves for 5-HT were very close to unity in several experiments.

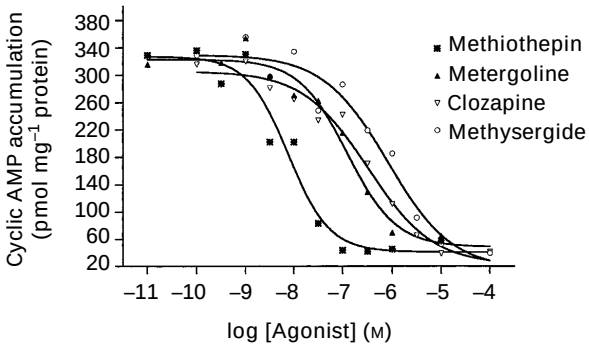

Figure 3 Antagonist-inhibition of 5-HT (1 μM)-stimulated cyclic AMP accumulation in 293 cells expressing the human 5-HT$_{7(b)}$ receptor. Shown is a single experiment; each curve is representative of at least three individual experiments. Hill slopes for the concentration-response curves for 5-HT were very close to unity. Thus, K_b values were calculated from the IC$_{50}$ values as described by Craig (1993).

Distribution of 5-HT$_7$ receptor splice variants

The expression patterns of 5-HT$_{7(a)}$ and 5-HT$_{7(b)}$ receptor mRNA were compared by PCR, with cDNAs from several human tissues (Table 4). The expression of the two splice variant receptors was similar in all tissues studied, although the 5-HT$_{7(b)}$ receptor mRNA was expressed at slightly lower levels in the small intestine, spleen and stomach. Whereas small

Table 1 Affinities (pK_i) and potencies (pEC$_{50}$, PK_B) of ligands for the recombinant (short) 5-HT$_{7(b)}$ receptor expressed in HEK 293 cells

Compounds	*Radioligand binding* (*pK_i*)	*CyclicAMP accumulation* (pEC$_{50}$)
Agonists		
5-CT	9.65±0.03	8.73±0.11
5-HT	9.41±0.04	7.54±0.13
5-MeOT	9.28±0.07	8.12±0.20
Dipropyl-5-CT	8.16±0.10	7.06±0.13
8-OH-DPAT	6.85±0.08	5.25±0.28
6-MeOT	–	4.99±0.06
Tryptamine	–	5.62±0.36
Antagonists		(pK_B)
Methiothepin	8.87±0.08	8.45±0.16
Metergoline	8.45±0.06	7.09±0.06
Pirenperone	8.19±0.08	7.72±0.17
Methysergide	7.57±0.13	6.66±0.23
Spiperone	7.65±0.08	6.03±0.12
Mesulergine	7.87±0.12	6.72±0.28
Mianserin	7.32±0.15	6.05±0.26
Clozapine	7.62±0.18	6.65±0.09
Haloperidol	6.30±0.11	–
Ritanserin	–	6.41±0.07
Ketanserin	6.44±0.17	5.30
Cyproheptadene	–	5.72±0.23
Prazosin	4.80±0.33	–
Pindolol	<5	–

Radioligand binding studies were performed in the presence of 0.3–0.7 nM [^{3}H]-5-CT. In binding isotherm studies with [^{3}H]-5-CT, K_d=0.28±0.06 nM, B_{max}=7.3±1.7 pmol mg^{-1} protein. For cyclicAMP accumulation studies, cells were stimulated for 20 min at 37°C. Antagonists were added to cells 10 min before agonist (5-HT) addition; pK_B values were calculated by use of the pEC$_{50}$ values for 5-HT-stimulated cyclicAMP accumulation.

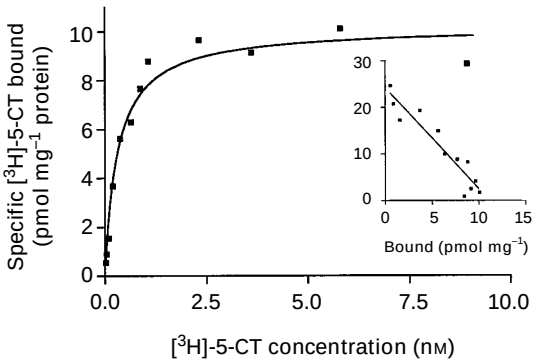

Figure 4 Radioligand ([^{3}H]-5-CT) binding isotherm at the human 5-HT$_{7(b)}$ receptor in 293 cell membranes. The data shown are representative of seven individual experiments. [^{3}H]-5-CT was found to have a pK_d=9.55±0.10, B_{max}=7.3±1.7 pmol mg^{-1} protein for this particular clone (#58).

amounts of 5-HT$_{7(a)}$ mRNA were detected in pancreas and kidney, expression of the 5-HT$_{7(b)}$ receptor could not be detected under the conditions used in our studies.

Discussion

Rat 5-HT$_7$ receptor cDNAs have been isolated from rat kidney proximal tubule (Shen *et al*., 1993) or brain (Ruat *et al*., 1993). The receptor described by Shen and colleagues (1993) encodes a 448 residue protein. In contrast, a 435 amino acid 5-HT$_7$ receptor, isolated from a rat brain cDNA library (Lovenberg *et al*., 1993), was identical to the other receptors with the exception of a truncated region at the carboxy-terminal tail of the receptor. Hence, the rat brain receptor contained a 5 base pair insertion at an intron splice site which introduced an 'in frame' stop codon, shortening the carboxy tail by 13 amino acids (Figure 1). An intron-containing h5-HT$_7$ receptor gene was first isolated from cDNA and genomic libraries and when expressed in cells, stimulated adenylyl cyclase with potencies consistent for a 5-HT$_7$ receptor (Bard *et al*., 1993). In the present study, we have identified a cDNA encoding a h5-HT$_7$ receptor, identical to that described by Bard *et al*. (1993), except that it possesses a truncated carboxy terminus. As illustrated for the rat sequences (Shen *et al*., 1993; Lovenberg *et al*., 1993), a thirteen amino acid truncation in the h5-HT$_7$ receptor results from a 5 bp insertion which introduces the stop codon (Figure 1).

Heidmann and colleagues (Heidmann *et al*., 1997) have recently demonstrated the presence of human and rat 5-HT$_7$ receptor isoforms. In addition to the 5-HT$_{7(b)}$ form described in our study, they have identified two other isoforms, one in the rat which they have named 5-HT$_{7(c)}$ and one in the human, designated 5-HT$_{7(d)}$. These two isoforms are generated from

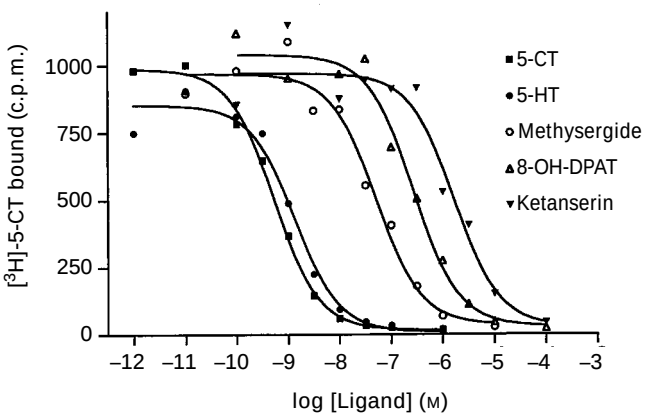

Figure 5 Antagonist-inhibition of [^{3}H]-5-CT binding in 293 cells expressing the human 5-HT$_{7(b)}$ receptor. Shown is a single experiment; each curve is representative of at least three individual experiments. K_i values were calculated from the IC$_{50}$ values, as described by Cheng & Prussoff (1973).

Table 2 Ligand affinity at the human 5-HT$_{7(b)}$ receptor expressed in different 293 cell clones

Compound	*Clone 5* (pK_i)	*Clone 12* (pK_i)	*Clone 58* (pK_i)
5-CT	9.57*±0.18	10.03±0.17	9.65±0.03
5-HT	9.38*±0.01	9.61±0.07	9.41±0.04
5-MeOT	9.41*±0.18	9.47±0.04	9.28±0.07
Dipropyl-5-CT	8.19±0.17	8.41±0.16	8.16±0.10
Methiothepin	9.00±0.18	9.20±0.10	8.87±0.08
Metergoline	8.65±0.04	8.86±0.02	8.45±0.06
Pirenperone	8.39*±0.15	8.53±0.12	8.19±0.08
Methysergide	7.75*±0.11	7.83±0.09	7.57±0.13
8-OH-DPAT	6.87±0.11	7.09±0.12	6.85±0.08
Spiperone	7.73*±0.05	7.96±0.13	7.65±0.08
Mesulergine	7.83*±0.13	8.10±0.03	7.87±0.12
Sumatriptan	5.88±0.09	5.97±0.05	5.71±0.12
Ketanserin	6.39*±0.25	6.53±0.19	6.44±0.17
Haloperidol	6.58±0.05	6.53±0.10	6.30±0.11
Pindolol	<5*±0.00	<5±0.00	<5±0.00
Mianserin	7.50±0.07	7.57±0.11	7.32±0.15
[^{3}H]-5-CT pK_d	9.66±0.19	9.89±0.04	9.55±0.10
B_{max} (pmol mg^{-1})	2.45±0.73	2.68±0.58	7.31±1.74

The affinity (pK_i) of a number of ligands was investigated in 293 cell membranes expressing the h5-HT$_{7(b)}$ receptor at varying receptor density. Data shown are mean±s.e.mean from 3–4 separate experiments except where noted (*n=2).

Table 3 Comparison of affinities (pK_i) of ligands at recombinant 5-HT$_7$ receptors from different species

Receptor	*h5-HT$_{7(a)}$*	*h5-HT$_{7(b)}$*	*h-5-HT$_{7(a)}$*	*r5-HT$_{7(a)}$*	*5-HT$_{7(b)}$*	*gp5-HT$_{7(a)}$*
Host cell	*HEK 293*	*HEK 293*	*Cos-7*	*CHO-K1*	*Cos-M6*	*CHO-K1*
Radioligand	*[^{3}H]-5-CT*	*[^{3}H]-5-CT*	*[^{3}H]-5-HT*	*[^{3}H]-5-HT*	*[^{125}I]-LSD*	*[^{3}H]-5-CT*
(Reference)	*Present*	*Present*	*(Bard* et al., *1993)*	*(Ruat* et al., *1993)*	*(Lovenberg* et al., *1993)*	*(To* et al., *1995)*
Agonists						
5-HT	9.7	9.4	8.1	9.2	8.1	9.6
5-CT	9.7	9.7	9.0	9.9	9.1	9.7
5-MeOT	9.4	9.3	8.3	9.0	–	9.3
8-OH-DPAT	6.9	6.9	6.3	7.3	7.0	7.4
Antagonists						
Methiothepin	9.0	8.9	8.4	–	8.9	8.4
Metergoline	8.5	8.5	8.2	7.2	7.5	8.2
Mesulergine	8.2	7.9	7.7	–	7.8	7.8
Clozapine	–	7.6	–	7.2	–	7.3
Cyproheptadine	–	–	6.9	7.1	–	6.9
Ritanserin	–	–	7.3	–	7.2	7.3
Methysergide	7.7	7.6	7.1	–	7.5	7.7
Spiperone	7.0	7.7	7.0	7.7	–	7.3
Mianserin	7.0	7.6	–	7.2	–	7.0
Ketanserin	5.9	6.4	5.9	<5.1	–	6.2

Data are the mean values from radioligand displacement experiments with recombinant receptors expressed in mammalian cells.

Table 4 Relative PCR quantitation distribution of h5Ht$_7$ receptor splice variant mRNA

Tissue	*5-HT$_{7(a)}$*	*5-HT$_{7b}$*
Amygdala	+ + + +	+ + + +
Aorta	+ + + +	+ + + +
Cerebral cortex	+ + +	+ + + +
Hippocampus	+ + + +	+ + +
Thalamus	+ + + +	+ + + +
Small Intestine	+ + + +	+ + +
Spleen	+ +	+
Pancreas	+	–
Stomach	+ +	+
Kidney	+	–
Liver	–	–

PCR was performed with a common 5′ primer and 3′ primers specific for each splice variant. Quantitation was by ethidium bromide staining ranges from a minimum of minus (undetectable) to a maximum of + + + +.

the use of two distinct exon cassettes located within intron 2 of the rat and human 5-HT$_7$ receptor gene sequence and result in gene products with carboxy-terminal amino acid sequences different from the 5-HT$_{7(a)}$ isoform as well as from each other. No pharmacological or functional studies on these isoforms were described for either the 470 amino acid rat 5-HT$_{7(c)}$ isoform or the 479 amino acid human 5-HT$_{7(d)}$ isoform in that study.

5-HT$_7$ receptors are discretely localized within the central nervous system of the guinea-pig and rat. In the former, 5-HT$_{7(a)}$ receptor protein and mRNA are localized primarily to corticolimbic systems of the brain (Tsou *et al*., 1994; To *et al*., 1995), with the highest density of *in situ* hybridization found in the pyramidal and granule cell layers of the hippocampus, periventricular thalamus and superficial cortex. Rat 5-HT$_{7(a)}$ receptor mRNA is expressed highly in the hypothalamus, hippocampus and brainstem, and at much lower levels in the stomach and ileum (Ruat *et al*., 1993) or spleen (Shen *et al*., 1993). Rat 5-HT$_{7(b)}$ receptor mRNA have only been detected in brain tissue, with highest levels in the thalamus and hypothalamus (Lovenberg *et al*., 1993). The h5-HT$_{7(a)}$ receptor is not only expressed in brain, but also in the gastrointestinal tract, kidney, liver, pancreas, spleen and coronary artery (Bard *et al*., 1993). Studies in endothelial and smooth muscle cells from human coronary and pulmonary artery and umbilical vein have also described 5-HT$_7$ receptor mRNA expression in smooth muscle cells (Ullmer *et al*., 1995; Schoeffter *et al*., 1996). In the present study, we have confirmed the expression pattern of h5-HT$_{7(a)}$ receptor mRNA and found it to be very similar to the expression of a 5-HT$_{7(b)}$ splice variant (Table 4). Like Heidmann *et al*. (1997), we found a higher level of h5-HT$_{7(a)}$ receptor mRNA in the spleen compared to the 5-HT$_{7(b)}$ receptor. Although small amounts of 5-HT$_{7(a)}$ mRNA were detected in pancreas and kidney, the 5-HT$_{7(b)}$ receptor was not detected in these tissues.

The 5-HT$_7$ receptor, from all species studied to date, positively couples to adenylyl cyclase (Plassat *et al*., 1993; Shen *et al*., 1993; Lovenberg *et al*., 1993; Bard *et al*., 1993; To *et al*., 1995). Accordingly, it was observed in the present study that the 5-HT$_{7(b)}$ receptor also augmented cyclic AMP accumulation in HEK 293 cells (Figure 2, Table 1). The rank order of agonist potency for this response was consistent with activation of the 5-HT$_7$ receptor. In this, as in other studies, a discrepancy was noted between the affinity of agonists, estimated in radioligand binding studies, and the potency at receptors mediating stimulation of adenylyl cyclase (5-HT, for example, displayed an affinity (pK_i) of 9.4 and a potency (pEC_{50}) of 7.5; Table 1). Furthermore, the affinities of antagonists at inhibiting 5-HT-stimulated cyclic AMP accumulation were somewhat lower than those seen for displacement of [^{3}H]-5-CT (Table 1). The reasons for these disparities are unclear but they have previously been noted in both recombinant and native 5-HT$_7$ systems (see Eglen *et al*., 1997, for review). It is possible that the receptor transiently exists in different conformational states when activating Gs proteins to those that may exist under conditions used in the binding assays, particularly since these are conducted over longer equilibrium periods. Clearly, additional work is required to elucidate the reason for these differences but these data highlight the necessity for identification of a high affinity, selective radioligand preferably derived from a silent surmountable antagonist.

To characterize further the h5-HT$_7$ receptor isoforms under similar conditions, both the h5-HT$_{7(a)}$ and 5-HT$_{7(b)}$ receptors were expressed in HEK 293 cells and ligand affinities were compared. The affinities of ligands were similar between the two splice variants (Table 3) and also with the h5-HT$_{7(a)}$ receptor expressed in Cos-7 cells (Table 3). Moreover, the affinity for 5-HT determined for both splice variants was similar to that obtained at the rat (pK_i = 9.2; Ruat *et al*., 1993) and guinea-pig 5-HT$_{7(a)}$ receptor (pK_i = 9.6; To *et al*., 1995). However,

for reasons that are unclear, a difference was noted between these data and those obtained by Bard *et al*. (1993), as illustrated by the affinity of 5-HT i.e. h5-HT$_{7(a)}$ pK_i = 9.7 (present study) and 8.1 (Bard *et al*., 1993).

Splice variations in the carboxy-terminus of other G-protein coupled receptors have been demonstrated, including prostanoid EP$_3$ receptors (Namba *et al*., 1993) and α_1-adrenoceptors (Hirasawa *et al*., 1995; Chang *et al*., 1997). Differences in the carboxy terminus of the EP$_3$ receptor have been suggested to direct the receptor variant to couple differentially to G-proteins (Namba *et al*., 1993; Kotani *et al*., 1995). In a similar fashion, directional coupling of 5-HT$_7$ receptor isoforms may permit differential regulation of cellular responses by a single neurotransmitter.

Nomenclature

The term, h5-HT$_{7(a)}$ appears to be appropriate to describe the receptor variant (long form), initially demonstrated by Bard and colleagues (1993) and the truncated variant described in the present study, as the h5-HT$_{7(b)}$ receptor, reflecting the order of its discovery. The recent study from Heidmann and colleagues (1996) supports the existence of truncated splice variants of the h5-HT$_7$ receptor and reveals an additional splice variant in the human (h5-HT$_{7(d)}$), and a different variant in the rat designated 5-HT$_{7(c)}$. Furthermore, since the 5-HT$_7$ receptor is well represented by several endogenous correlates in the brain and periphery (see Eglen *et al*., 1997, for review), the receptor is designated the upper case appellation i.e. h5-HT$_{7(a)}$, h5-HT$_{7(b)}$ or h5-HT$_{7(d)}$. It should be noted that the receptors, nonetheless, still lack selective agonists or antagonists, and, indeed, these isoforms are operationally very similar.

In summary, the h5-HT$_7$ receptor, like the rat receptor, exists in multiple isoforms. While the physiological roles of these splice variants, remain to be clarified, both the h5-HT$_{7(a)}$ and h5-HT$_{7(b)}$ receptor isoforms couple positively to adenylyl cyclase and possess similar distribution patterns and pharmacology. It will be interesting to compare the pharmacological characteristics of the h5-HT$_{7(a)}$ and h5-HT$_{7(b)}$ receptors with the newly described h5-HT$_{7(d)}$ receptor (Heidmann *et al*., 1997).

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