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Structure and functional expression of cloned rat serotonin 5HT-2 receptor

Dolan B.Pritchett, Alfred W.J.Bach[1], Manfred Wozny[2], Omar Taleb, Roberto Dal Toso, Jean C.Shih[3] and Peter H.Seeburg

Laboratory of Molecular Neuroendocrinology, University of Heidelberg, ZMBH, INF 282, D-6900 Heidelberg, [2]BASF, AG., Abt. Biotechnologie, Carl-Bosch-Str. 38, D-6700 Ludwigshafen, FRG and [3]Division of Biological Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA 90033, USA

[1]Present address: BASF, AG., Abt. Biotechnologie, Carl-Bosch-Str. 38, D-6700 Ludwigshafen, FRG

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A complementary DNA (cDNA) encoding a serotonin receptor with 51% sequence identity to the 5HT-1C subtype was isolated from a rat brain cDNA library by homology screening. Transient expression of the cloned cDNA in mammalian cells was used to establish the pharmacological profile of the encoded receptor polypeptide. Membranes from transfected cells showed high-affinity binding of the serotonin antagonists spiperone, ketanserin and mianserin, low affinity for haloperidol (a dopamine D2 receptor antagonist), 8-OH-DPAT as well as MDL-72222 and no detectable binding of [^{3}H]serotonin. This profile is consonant with the 5HT-2 subtype of serotonin receptors. In agreement with this assignment, serotonin increased the intracellular Ca^{2+} concentration and activated phosphoinositide hydrolysis in transfected mammalian cells. The agonist also elicited a current flow, blocked by spiperone, in *Xenopus* oocytes injected with *in vitro* synthesized RNA containing the cloned nucleotide sequences.

Key words: Ca mobilization/inositolphosphates/mammalian cell expression/oocyte expression/receptor subtypes

Introduction

Serotonin (5-hydroxytryptamine, 5HT) was first discovered as a serum factor and later shown also to be a neurotransmitter in the central and peripheral nervous systems (reviewed by Leysen, 1988). It interacts with a family of pharmacologically distinct receptors on the surface of neurons and other cells in the body (reviewed by Peroutka, 1988). These receptors have been grouped into three major classes (5HT-1, 5HT-2 and 5HT-3) based on interactions with various agonists and antagonists (Bradley *et al.*, 1986). The first class contains at least four subtypes (5HT-1A–5HT-1D). The primary structure of a 5HT-1C receptor was recently described (Julius *et al.*, 1988) and characterizes a new member of the G-protein-coupled receptor family containing seven transmembrane regions (Lefkowitz *et al.*, 1986). This receptor is located primarily in the choroid plexus, and its functional role there is unknown.

In contrast to the 5HT-1 class, 5HT-2 sites seem to be homogeneous (Leysen *et al.*, 1981). The highest level of 5HT-2 binding in brain is seen in layer IV of the cerebral cortex (Pazos *et al.*, 1985). A number of central (Glennon *et al.*, 1984) and peripheral effects of 5HT have been attributed to this subtype. Thus, 5HT-elicited seizures can be prevented by 5HT-2-receptor-specific antagonists (Leysen *et al.*, 1978). This, and its involvement in tracheal smooth muscle contraction, bronchoconstriction (Leysen *et al.*, 1984) and control of aldosterone production (Matsuoka *et al.*, 1985) make it desirable to describe the structure of this receptor. Because both the 5HT-1C and 5HT-2 subtypes appear to exert their cellular effects through phosphoinositol turnover, they may be structurally closely related in analogy to the M1 and M4 muscarinic acetylcholine receptor subtypes (Peralta *et al.*, 1988). Using oligonucleotides encoding 5HT-1C sequences, we have isolated a cloned cDNA encoding the complete 5HT-2 receptor. This report describes the structure as well as the pharmacology and functional coupling of this receptor.

Results

Receptor cloning

Two oligonucleotides directed against amino acid residues 88–104 and 134–149 of the 5HT-1C receptor (Julius *et al.*, 1988) were used to probe a cloned cDNA library constructed in λgt10 from rat forebrain mRNA. Several recombinant phages were identified and the cloned cDNAs analysed by nucleotide sequencing. One cDNA specified an open reading frame of 449 amino acids preceded by 668 nucleotides of 5′ untranslated sequence (Figure 1). The predicted polypeptide contains seven putative transmembrane regions, typical of G-protein-coupled receptors. The amino acid sequence is 51% identical to that of the 5HT-1C receptor. Highest identity is seen in the transmembrane regions (one of these, the fifth, is identical in both receptors), and lowest sequence matches occur in the N-terminal 60 amino acid residues, the intracellularly located loop between transmembrane regions V and VI, as well as in the C-terminus. Such a pattern of sequence identity is also observed between different muscarinic acetylcholine receptors (Bonner *et al.*, 1987; Peralta *et al.*, 1987) and suggests that the cloned cDNA encodes a 5HT-receptor subtype. A sequence alignment with the 5HT-1C receptor and several other class II neurotransmitter receptors is shown in Figure 2.

Receptor pharmacology

To determine the pharmacological profile of the receptor encoded by the cloned cDNA, we expressed this cDNA transiently in a mammalian cell line by transfection with a recombinant plasmid DNA containing the entire coding region of the cDNA under the control of a cytomegalovirus promoter (Gorman *et al.*, 1983; Peralta *et al.*, 1987). Membranes prepared from these cells were analysed by binding of a variety of 5HT-receptor ligands. As shown in Figure 3a, high-affinity sites (0.5 ± 0.1 nM) were present

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   1 ACGCGATAACTGTCAATATGAAAGTCACTTGATTTTTACAGAAATACAAGCTCAAATTCCTGGACCGCGGCTGAGGCACTTCTGCCTGAG
  91 ACTAAGAAGGGTTAACCCAAGAATATTCAGTTAGGATGGCATTAACACTGTGTAGGTTTTTAATTGACTTCCTTAATTGATATAGAGGGC
 181 ACACTGCCTCCCTCCTCGTTTGGATCTCATGCTGTTTTAACTTTGTGATGGCTGAACTCTTGGAAGCAGCATATTCAACCCGAGAATTAG
 271 CTGAAAGATTTTCACGGATACAAAACTTTTCTTCCTTGAACCAGGAACACGTTTGTGTCCCCGAATACTCAAAGTGCTTTTTTTGGCCTT
 361 TGCTTCCGTGAGAACTTACAGCTCAGCCGTGGGCTCTCCCTAGCACCGTGAAGGGAGGCATAATCAAGAAGCATCACACTTCTGTAACTC
 451 TTACTATGGAAGAGGAGAAGGCAGCCAGAGGAGCCACACATGTTCTCCGCTTCAGCACGTCCTAGCGCCAGGGCACGAAGATGAATGGTG
 541 AGCCCAGGCTATGACCCCCTAGTCTCTCCACACTTCATCTGCTACAACTTCCGGCTTAGACATGGAAATTCTTTGTGAAGACAATATCTC
 631 TCTGAGCTCAATTCCAAACTCCTTAATGCAATTAGGTGATGGCCCGAGGCTCTACCATAATGACTTCAACTCCAGAGATGCTTAACACTT
   1                                   M  A  R  G  S  T  I  M  T  S  T  P  E  M  L  N  T
 721 CGGAAGCATCGAACTGGACAATTGATGCTGAAAACAGAACCAACCTCTCCTGTGAAGGGTACCTCCCACCGACATGCCTCTCCATTCTTC
  18 S  E  A  S  N  W  T  I  D  A  E  N  R  T  N  L  S  C  E  G  Y  L  P  P  T  C  L  S  I  L
 811 ATCTCCAGGAAAAAAACTGGTCTGCTTTATTGACAACTGTCGTGATTATTCTCACCATTGCTGGAAATATACTGGTCATCATGGCAGTGT
  48 H  L  Q  E  K  N  W  S  A  L  L  T  T  V  V  I  I  L  T  I  A  G  N  I  L  V  I  M  A  V
 901 CCCTAGAAAAAAAGCTGCAGAATGCCACCAACTATTTCCTGATGTCACTTGCCATAGCTGATATGCTGCTGGGTTTCCTTGTCATGCCTG
  78 S  L  E  K  K  L  Q  N  A  T  N  Y  F  L  M  S  L  A  I  A  D  M  L  L  G  F  L  V  M  P
 991 TGTCCATGTTAACCATCCTGTATGGGTACCGGTGGCCTTTGCCTAGCAAGCTCTGTGCGATCTGGATTTACCTGGATGTGCTCTTTTCTA
 108 V  S  M  L  T  I  L  Y  G  Y  R  W  P  L  P  S  K  L  C  A  I  W  I  Y  L  D  V  L  F  S
1081 CGGCATCCATCATGCACCTCTGCGCCATCTCCCTGGACCGCTATGTCGCCATCCAGAACCCCATTCACCACAGCCGCTTCAACTCCAGAA
 138 T  A  S  I  M  H  L  C  A  I  S  L  D  R  Y  V  A  I  Q  N  P  I  H  H  S  R  F  N  S  R
1171 CCAAAGCCTTCCTGAAAATCATTGCCGTGTGGACCATATCTGTAGGTATATCCATGCCAATCCCAGTCTTTGGACTACAGGATGATTCGA
 168 T  K  A  F  L  K  I  I  A  V  W  T  I  S  V  G  I  S  M  P  I  P  V  F  G  L  Q  D  D  S
1261 AGGTCTTTAAGGAGGGGAGCTGCCTGCTTGCCGATGACAACTTGTTCTCATAGGCTCTTTTGTGGCATTTTTCATCCCCCTAACCATCA
 198 K  V  F  K  E  G  S  C  L  L  A  D  D  N  F  V  L  I  G  S  F  V  A  F  F  I  P  L  T  I
1351 TGGTGATCACCTACTTCCTGACTATCAAGTCACTTCAGAAAGAAGCCACCTTGTGTGTGAGTGACCTCAGCACTCGAGCCAAACTAGCCT
 228 M  V  I  T  Y  F  L  T  I  K  S  L  Q  K  E  A  T  L  C  V  S  D  L  S  T  R  A  K  L  A
1441 CCTTCAGCTTCCTCCCTCAGAGTTCTCTGTCATCAGAAAAGCTCTTCCAACGGTCCATCCACAGAGAGCCAGGCTCCTACGCAGGCCGAA
 258 S  F  S  F  L  P  Q  S  S  L  S  S  E  K  L  F  Q  R  S  I  H  R  E  P  G  S  Y  A  G  R
1531 GGACGATGCAGTCCATCAGCAATGAGCAAAAGGCGTGCAAGGTGCTGGGCATCGTGTTCTTCCTGTTTGTTGTAATGTGGTGCCCATTCT
 288 R  T  M  Q  S  I  S  N  E  Q  K  A  C  K  V  L  G  I  V  F  F  L  F  V  V  M  W  C  P  F
1621 TCATCACCAATATCATGGCCGTCATCTGCAAAGAATCCTGCAATGAAAATGTCATCGGAGCCCTGCTCAATGTGTTTGTCTGGATTGGTT
 318 F  I  T  N  I  M  A  V  I  C  K  E  S  C  N  E  N  V  I  G  A  L  L  N  V  F  V  W  I  G
1711 ATCTCTCCTCAGCTGTCAATCCACTGGTATATACGTTGTTCAATAAAACTTATAGGTCCGCCTTCTCAAGGTACATTCAGTGTCAGTACA
 348 Y  L  S  S  A  V  N  P  L  V  Y  T  L  F  N  K  T  Y  R  S  A  F  S  R  Y  I  Q  C  Q  Y
1801 AGGAAAACAGAAAGCCACTGCAGTTAATTTTAGTGAACACTATACCAGCATTGGCCTACAAGTCTAGTCAGCTCCAGGTGGGACAGAAAA
 378 K  E  N  R  K  P  L  Q  L  I  L  V  N  T  I  P  A  L  A  Y  K  S  S  Q  L  Q  V  G  Q  K
1891 AGAACTCACAGGAAGATGCTGAGCAGACAGTTGATGACTGCTCCATGGTTACACTGGGGAAACAACAGTCGGAAGAGAATTGTACAGACA
 408 K  N  S  Q  E  D  A  E  Q  T  V  D  D  C  S  M  V  T  L  G  K  Q  Q  S  E  E  N  C  T  D
1981 ATATTGAAACCGTGAATGAAAAGGTTAGCTGTGTGTGATGAACTGGATGCTATGGCAATTGCCAGGGCATGTGAACAAGGTTATACCCAT
 438 N  I  E  T  V  N  E  K  V  S  C  V
2071 GTGTGTGGGGCGGGGATAAGGAGGCTGCAACAAATTAGACTACTCCAGTCGACCAACGTATAATGTCCTGACAGCATTGGGAGCTAGGAG
2161 TGTTACGATGCTTTAATTATTGCCAATGAGATCTCTAAAACCATTGGCCCGTCTCGA
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of the rat 5HT-2 receptor. Numbering of nucleotides and amino acid residues is shown on the left. Amino acids appear in single-letter code.

for spiperone, a 5HT-2 and dopamine D2 receptor antagonist. No such sites were detected on untransfected cells. The affinity of spiperone for our receptor is three orders of magnitude higher than the reported affinity for the 5HT-1B, 5HT-1C and 5HT-1D receptors and two orders of magnitude higher than for the 5HT-1A subtype (Peroutka, 1986; Leysen, 1988), suggesting that our cloned cDNA encodes a 5HT-2 receptor or the dopamine-D2-receptor subtype. Consistent with this conclusion [^{3}H]5HT, which should bind to the 5HT-1A, 5HT-1B and 5HT-1D subtypes (Peroutka, 1986), did not show any detectable binding to the expressed receptor.

Using ligand competition (Figure 3b), the 5HT antagonists ketanserin and mianserin displayed affinities of 1.0 ± 0.5 and 1.8 ± 0.3 nM respectively, in close agreement with the values and rank orders expected for their interaction with the 5HT-2 receptor (Leysen *et al.*, 1982; Hoyer *et al.*, 1985). The observed K_i of ketanserin for this receptor is two orders of magnitude lower than the K_i for the 5HT-1C receptor. The high affinities of mianserin and ketanserin exclude the possibility that the cloned cDNA encodes the D2 receptor. In fact, haloperidol, a dopamine D2 receptor antagonist, displayed a K_i of 125 nM, in high excess of the 1.4 nM expected for the D2 receptor (Leysen *et al.*, 1982) and only slightly higher than the value reported for the cortical 5HT-2 receptor (Leysen *et al.*, 1981). The agonist 5HT itself displaced spiperone with a K_i of 250 nM, 4-fold lower than reported for the 5HT-2 receptor (Leysen *et al.*, 1981). 8-OH-DPAT had an extremely low affinity consistent with the known affinity for the 5HT-2 subtype (Hoyer *et al.*, 1985). The 5HT-3 receptor antagonist MDL-72222 (10^{-6} M) was ineffective in competition binding studies (data not shown). This eliminates the possibility that the cloned cDNA encodes the 5HT-3 receptor (Hoyer and Neijt, 1988), which is thought to be a ligand-gated ion channel (Hoyer, 1988). Thus, the results of our ligand-binding studies clearly demonstrate that the cDNA encodes a 5HT-2 receptor. This 5HT-receptor subtype is known for its high affinity to antagonists and low affinity to agonists (Leysen, 1988).

Effector coupling to PI hydrolysis in Ca release

5HT-induced phosphoinositide (PI) hydrolysis appears to occur as a result of 5HT-2 receptor activation in cortex (Kendall and Nahorski, 1985), thoracic aorta (Roth *et al.*, 1984), and platelets (De Chaffoy *et al.*, 1985). The same second messenger system is stimulated by the 5HT-1C receptor and may modulate a number of cellular processes, including Ca^{2+} release from intracellular stores and protein kinase C activation. To determine if the cloned receptor is coupled to this pathway, we analysed serotonin-triggered changes in the levels of intracellular Ca^{2+} using the Ca^{2+}-sensitive dye FURA-2/AM (Grynkiewicz *et al.*, 1985). This dye undergoes changes in spectral properties in response to the concentration of free Ca^{2+}. As shown in Figure 4, agonist-dependent intensity shifts were seen in cells expressing the 5HT-2 receptor. In untransfected cells or in transfected cells expressing the muscarinic receptor M4 subtype (Braun *et al.*, 1987), no change in intracellular Ca^{2+} concentration could be detected, using 5HT at

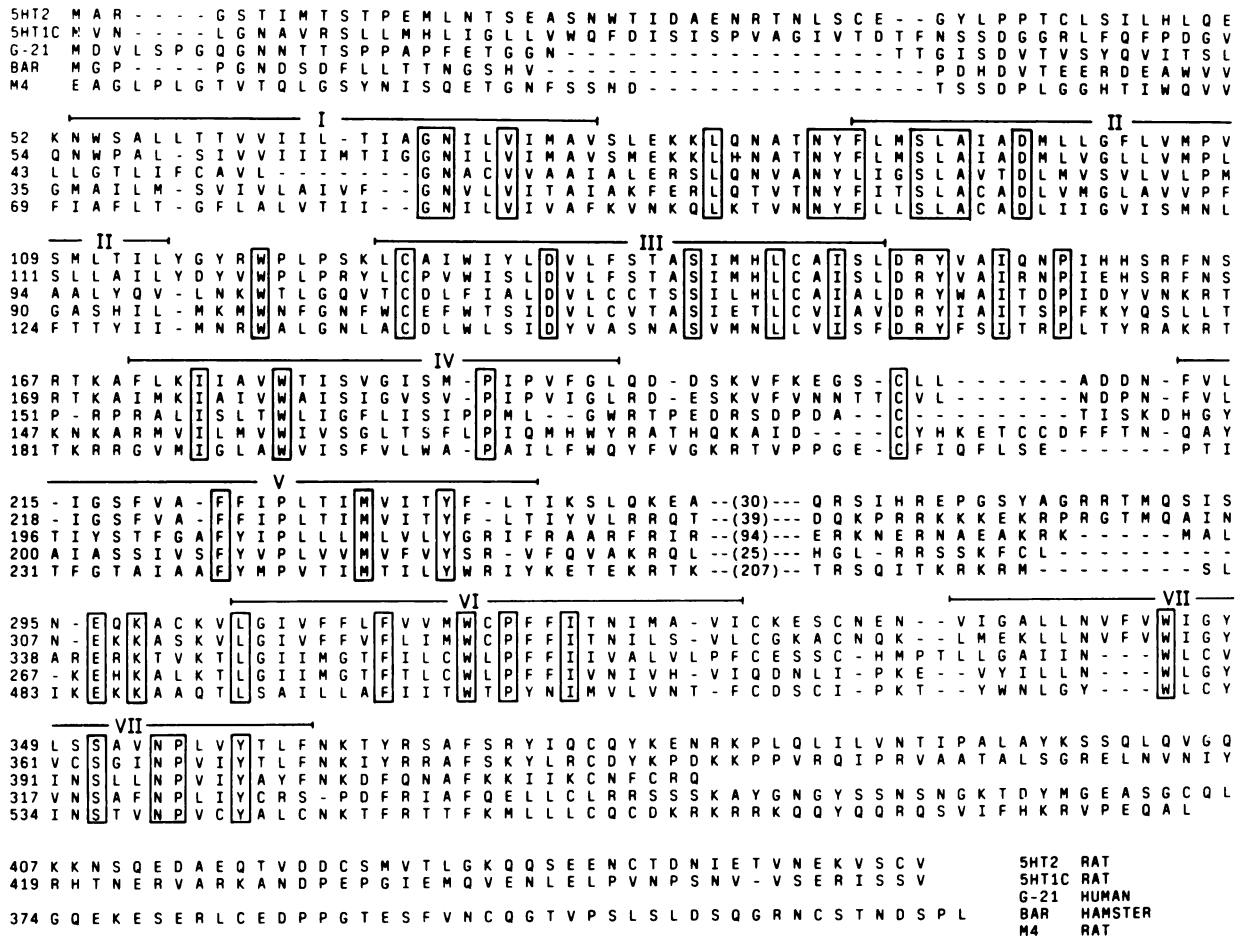

Fig. 2. Alignment of the primary structure of the 5HT-2 receptor with the amino acid sequences of several G-protein-coupled receptors: rat 5HT-1C (Julius *et al.*, 1988), human G-21 (Kobilka *et al.*, 1987) which is a serotonin 5HT-1A subtype (Fargin *et al.*, 1988), hamster β_2-adrenergic (Dixon *et al.*, 1986), and rat muscarinic acetylcholine M4 subtype (Braun *et al.*, 1987). Identical amino acids in all receptors are boxed, putative transmembrane segments are overlined. The residue numbers for each receptor are given at the beginning of each line.

10^{-4} M (data not shown). Based on the observed intensity shifts, intracellular free Ca^{2+} was routinely raised at least 3-fold at 10^{-7} M 5HT. The effect of 5HT could be blocked completely by 10 nM spiperone and was significantly reduced by 3 nM of the antagonist. Cells transfected with the rat M4 receptor cDNA responded in a similar manner to the agonist carbachol at saturating (10^{-4} M) concentrations (data not shown).

Direct measurement of PI hydrolysis in response to saturating levels of 5HT was shown by kinetic analysis of the accumulation of inositol mono-, bis- and trisphosphates (IP_1, IP_2 and IP_3) in transfected cells. In the presence of 10 mM LiCl, IP_1 levels increased linearly in the transiently transfected cells for 30 min following agonist exposure (10^{-5} M). This increase resulted in a >10-fold stimulation of IP_1 (data not shown). IP_2 and IP_3 levels increased 4-fold and 1.5-fold respectively. All increases were blocked (50%) by 10 nM spiperone. These results demonstrate that the cloned cDNA encodes a 5HT receptor which functionally activates phospholipase C and substantially elevates free Ca^{2+} levels. The activation of PI turnover strongly suggests that the receptor is a 5HT-2 subtype, in excellent agreement with its pharmacological profile.

5HT-2 receptor expression in Xenopus oocytes

In vitro generated RNA having the same nucleotide sequence as the cloned cDNA and encoding the 5HT-2 receptor was injected into *Xenopus* oocytes. In these oocytes serotonin (10^{-5} M) evoked fast desensitizing inward currents of several hundred nA when recorded under voltage clamp conditions at a holding potential of −70 mV. No response to serotonin was seen in uninjected oocytes or oocytes injected with antisense 5HT-2 RNA. The current−voltage relationship for the RNA-induced 5HT response was linear and showed a reversal potential of −27 mV (data not shown) which corresponds to the equilibrium potential of chloride in *Xenopus* oocytes (Dascal *et al.*, 1984). Superfusion with 10 nM spiperone greatly reduced 5HT-evoked currents (Figure 5). Only small effects on 5HT-1C-receptor activity were reported, using high concentrations (10^{-6} M) of spiperone (Julius *et al.*, 1988). Our results demonstrate that the 5HT-2 receptor can activate a second messenger system in oocytes, which controls Ca^{2+}-sensitive chloride channels by Ca^{2+} released from IP_3-sensitive intracellular stores (Takahashi *et al.*, 1987). For 5HT receptors, the activation of these channels has been reported previously only for the 5HT-1C subtype (Lübbert *et al.*, 1987b; Julius *et al.*, 1988).

Discussion

The structure, pharmacology and functional coupling of the receptor encoded by the cloned cDNA indicate that this is a 5HT-2 subtype of serotonin receptors. Thus, its design shows the presence of seven putative transmembrane regions,

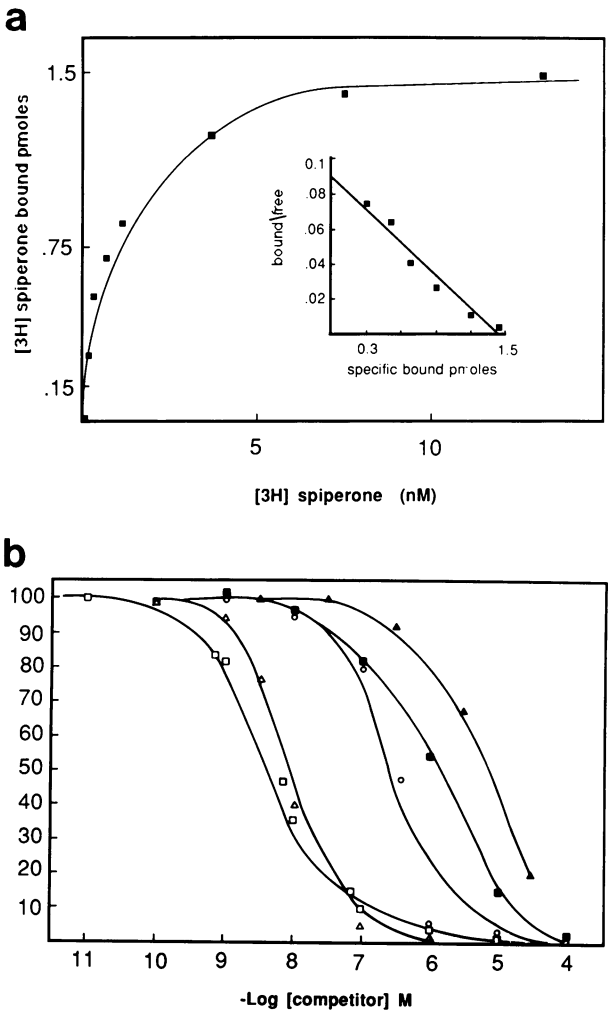

Fig. 3. Pharmacological profile of the 5HT-2 receptor in transiently transfected mammalian cells. (**a**) Binding isotherm of [^{3}H]spiperone to cell membranes. Inset: Scatchard plot of [^{3}H]spiperone binding. The K_d derived from this and two other experiments was 0.5 ± 0.1 nM. B_{max} values were dependent upon the efficiency of transfection and averaged 10 000 receptors/cell. (**b**) Competition binding of antagonists and agonists. The binding of [^{3}H]spiperone (1 nM) was assayed in the presence of different concentrations of competing ligands (ketanserin, □; mianserin, △; haloperidol, ○; 5HT, ■; 8-OH-DPAT, ▲). The assays were conducted as described in Materials and methods.

typical of G-protein-coupled neurotransmitter receptors (class II receptors). These seven regions together form the ligand-binding domain in receptors of this class. Of all the known members of this class, the structure of the described receptor is closest to that of the 5HT-1C receptor (Julius *et al.*, 1988). The affinities of known 5HT receptor antagonists and agonists closely match the profile expected for the 5HT-2 receptor from cortical preparations. Some minor discrepancies were observed, notably the higher than expected 5HT affinity and the lower affinity of the dopamine D2 receptor antagonist haloperidol. These discrepancies may reflect the lack of cross-reacting receptor types in the mammalian cell expression system, as well as the much higher specific to non-specific binding ratio of ligands than that seen in tissue preparations. However, it is also possible that these discrepancies define one of several 5HT-2 subtypes. Although the minor pharmacological differences do not

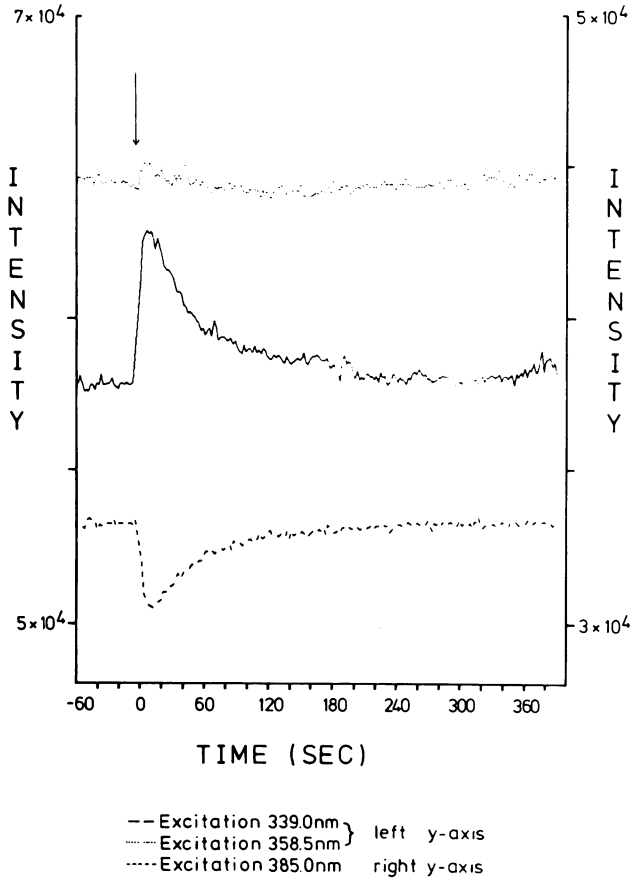

Fig. 4. Rise in intracellular Ca^{2+} concentration in response to the application (indicated by arrow) of 5HT at a final concentration of 10^{-7} M. After loading of transfected cells with the Ca^{2+}-sensitive dye FURA-2, the release of intracellular free Ca^{2+} was monitored by the intensity change of the fluorescence FURA-2. Numbers on *y*-axis denote detected photons per second. The intensity increased at 339 nm, decreased at 385 nm and was unchanged at 358.5 nm, as expected for Ca^{2+} induced changes of FURA-2 fluorescence.

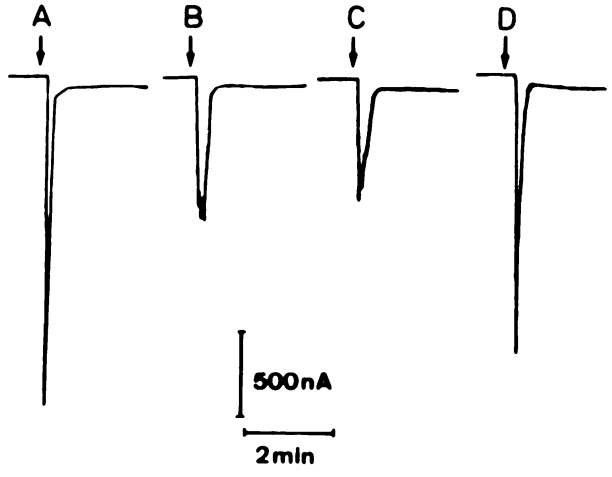

Fig. 5. Current traces recorded from *Xenopus* oocytes injected with *in vitro* synthesized RNA encoding the 5HT-2 receptor. Oocytes were clamped at −70 mV. A fast desensitizing inward current is seen, in response to the application of 5HT, reversibly reduced by spiperone. Applications were: 10^{-5} M 5HT (A); 10^{-5} M 5HT and 10^{-8} M spiperone (B and C); 10^{-5} M 5HT, after spiperone washout for 15 min (D).

prove the existence of more than one 5HT-2 receptor, the ongoing isolation of new muscarinic acetylcholine receptors using recombinant DNA technology (Bonner *et al.*, 1988) may serve as an example for unexpected subtype heterogeneity.

Consistent with the observed activation of phospholipase C in mammalian cells, the 5HT-2 receptor activates a chloride channel in *Xenopus oocytes* (Nomura *et al.*, 1987). This finding was not anticipated from existing literature, where only 5HT-1C receptor expression had been reported, using injections of brain RNA (Lübbert *et al.*, 1987a; Julius *et al.*, 1988). There are several explanations for this described lack of 5HT-2 receptor response. The corresponding mRNA may be of lower abundance than 5HT-1C mRNA or may be less efficiently translated. The 5HT-1C receptor might couple better to the relevant oocyte G protein than does the 5HT-2 receptor. Finally, the two receptors might differ in their respective rates of desensitization, as suggested by the fact that high concentrations of 5HT cause a desensitization in oocytes injected with brain RNA, but not in oocytes injected with choroid plexus RNA (Lübbert *et al.*, 1987a). Further experiments are needed to evaluate these possibilities.

Of all the known class II receptors, the 5HT-2 receptor is closest in structure to the 5HT-1C receptor. The high sequence identity (51%) between the 5HT-2 and the 5HT-1C receptors proves their structural similarity, suggested previously on the basis of pharmacological profiles and second messenger studies (Hoyer, 1988). The close structural and functional relationship between these two 5HT receptors suggests that they should be grouped by nomenclature (e.g. 5HT-2A and 5HT-2B). Such a change in nomenclature would also reflect the more distant structural relationship between 5HT-1C- and 5HT-1A-receptor subtypes. Thus, the latter receptor is encoded by the cloned G-21 gene (Fargin *et al.*, 1988) and shows only 35% sequence identity to the 5HT-1C and 5HT-2 receptors.

Materials and methods

Materials

Pharmacological agents were obtained from Janssen Pharmaceutica or Research Biochemicals International. Other chemicals were from Sigma or Merck. DNA-modifying enzymes were obtained from Boehringer Mannheim or New England Biolabs and fluorescent dyes were purchased from Molecular Probes (Junction City, OR, USA). Oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer model 380B.

Isolation of cloned 5HT-2 receptor cDNA

A rat forebrain cDNA library was screened at moderate stringency (hybridization at 30% formamide, 5 × SSC where 1 × SSC = 0.15 M NaCl/0.015 M sodium citrate, 37°C. Filters were washed at 55°C, 0.5 × SSC) using two synthetic oligonucleotides as probes. Oligonucleotide sequences encoded 5HT-1C receptor residues 88–104 (5′GATGGCGCAC-AGGTGCATGATGGAGGCGGTGGAGAACAGCACGTC3′) and residues 134–149 (5′GCCACCAACTACTTCCTGATGTCCCTGGCCA-TTGCTGACATGCTGGTGGG3′) (Julius *et al.*, 1988). DNA was prepared from recombinant λgt10 phage which hybridized to both probes. The cloned cDNA inserts were excised by digestion with *Eco*RI and subcloned into M13 phage vectors. DNA was sequenced by the chain termination method (Sanger *et al.*, 1977).

Cell culture and transfection

Human embryonic kidney 293 cells (ATCC) were grown in MEM supplemented with 10% fetal calf serum. For transfection, these cells were plated at 1–3 × 10^6 cells/10-cm plate and were used for transfection at 50% confluency. *Sac*I–*Eco*RI restriction fragment of the cloned cDNA (~1600 bp and containing the complete coding region) was cloned into the *Hpa*I restriction site of an expression vector plasmid (Peralta *et al.*, 1987; Pritchett *et al.*, 1988). DNA of this construct was prepared and purified by equilibrium centrifugation banding on a caesium chloride gradient. The purified DNA was used to transfect exponentially growing 293 cells, using the calcium phosphate method as modified by Chen and Okayama (1986). The precipitate was washed off after 16–20 h and fresh growth medium was added. The cells were allowed to grow up to 48 h from the time the DNA precipitate was added.

Membrane preparation and binding assay

Cells were harvested by centrifugation after aspiration in cold phosphate-buffered saline (PBS), lacking Ca^{2+} and Mg^{2+}. The cell pellet was washed twice in PBS. Cells were homogenized and membranes prepared according to Peroutka (1986) or frozen at −70°C up to 48 h before homogenization. The final assay volume was 1 ml and contained 0.1 ml of [^{3}H]spiperone and 0.1 ml of competing ligands. The ligands were dissolved in ethanol at 1 mM and diluted in homogenization buffer. After a 30-min incubation the triplicate samples were suction-filtered rapidly over Whatman GFB filters and washed twice with 50 mM Tris, pH 7.6. The radioactivity in the samples was determined and the data analysed by the linear least squares method.

FURA-2 measurements

Ca^{2+}-sensitive dye FURA-2 acetoxymethyl ester at a final concentration of 4 × 10^{-6} M was added to cells 48 h after transfection. The cells were dye-loaded for 30 min at 37°C. After three washes in calcium-free PBS, 10^6 cells were suspended in 2 ml of PBS and loaded into a cuvette containing a small magnetic stir bar. Agonist-induced dye fluorescence was measured in a SPEX photon-counting spectrofluorometer. PBS controls were added first, followed by the addition of the ligands dissolved in PBS. The emission was measured at 500 nm. Slit widths of the emission and excitation monochrometer were 15 and 6 nm respectively. The intensity signal remained constant (<1% change) during the course of measurements. Concentrations of Ca^{2+} were calculated from the excitation spectra for FURA-2 (Grynkiewicz *et al.*, 1985).

Phosphoinositol turnover assay

Assays on transfected cells were performed as described by Peralta *et al.* (1988). Briefly, 2 × 10^{-6} μCi/ml of [^{3}H]myo-inositol (NEN) were added to the growth media 16 h after transfection. After 32 h, the cells were washed three times in serum-free media and incubated in this medium for an additional 45 min. Media were removed, and serum-free medium containing 10 mM LiCl was added for 15 min. This medium was again removed, and the cells were suspended in serum-free medium containing 10 mM LiCl at 2 × 10^6 cells/ml. After addition of the agonist or antagonist to 0.5-ml cell suspension, the incubation was terminated by the addition of 1 ml of 15% trichloric acid. After three ether extractions and neutralization with 6 mM borax, the samples were chromatographed on Dowex AG1×8 columns (Biorad) in the formate form. Elution of IP_1, IP_2 and IP_3 was as follows: 10 ml H_2O (wash), 10 ml of 5 mM borax/60 mM Na-formate (wash), 10 ml of 0.1 M formic acid/0.2 M NH_4-formate (IP_1), 10 ml of 0.1 M formic acid/0.4 M NH_4-formate (IP_2) and 10 ml of 0.1 M formic acid/1.0 M NH_4 formate (IP_3). The radioactivity in 2 ml of each eluate was determined after the addition of 10 ml of scintillation fluid.

Oocyte injection and current recordings

After surgical removal, oocytes from *Xenopus laevis* were dissected and collagenase treated for 2 h to obtain single defolliculated oocytes. The cells at maturation stage IV were selected and pressure injected with RNA (25 ng in 100 nl), using pipettes with a tip of 10–15 μm diameter. Injected oocytes were incubated at 20°C in Barth's medium containing penicillin and streptomycin (100 U/ml). Voltage clamp experiments on these oocytes were performed 3 days after injection. The cells were held in a recording chamber (volume ~0.4 ml), using a two-electrode voltage clamp amplifer (Takahashi *et al.*, 1987) and perfused at 4 ml/min with frog Ringer solution (115 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 10 mM Hepes, pH 7.2). The current injection and voltage recording microelectrodes were filled with 3M KCl. 5HT and spiperone were applied by manual switching of the perfusion lines.

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References

Bonner,T.I., Buckley,N.J., Young,A.C. and Brann,M.R. (1987) *Science*, **237**, 527–532.

Bonner,T.I., Young,A.C., Brann,M.R. and Buckley,N.J. (1988) *Neuron*, **1**, 403–410.

Bradley,P.B., Engel,G., Feniuk,W., Fozard,J.R., Humphrey,P.P.A., Middlemiss,D.N., Mylecharane,E.J., Richardson,B.P. and Saxena,P.R. (1986) *Neuropharmacology*, **25**, 563–576.

Braun,T., Schofield,P.R., Shivers,B.D., Pritchett,D.B. and Seeburg,P.H. (1987) *Biochem. Biophys. Res. Comm.*, **149**, 125–132.

Chen,C. and Okayama,H. (1987) *Mol. Cell. Biol.*, **7**, 2745–2751.

Dascal,N., Landan,E.M. and Lass,Y. (1984) *J. Physiol.*, **352**, 551–574.

de Chaffoy de Courcelles,D., Leysen,J.E., De Clerck,F., Van Belle,H. and Janssen,P.A.J. (1985) *J. Biol. Chem.*, **260**, 7603–7608.

Dixon,R.A.F. *et al.* (1986) *Nature*, **321**, 75–79.

Fargin,A., Raymond,J.R., Lohse,M.J., Kobilka,B.K., Caron,M.G. and Lefkowitz,R.J. (1988) *Nature*, **335**, 358–360.

Glennon,R.A., Titeler,M. and McKenney,J.D. (1984) *Life Sci.*, **35**, 2505–2511.

Gorman,C., Padmanabram,R. and Howard,B. (1983) *Science*, **221**, 551–553.

Grynkiewicz,G., Poenie,M. and Tsien,R.Y. (1985) *J. Biol. Chem.*, **260**, 3440–3450.

Hoyer,D. (1988) *Trends Pharmacol. Sci.*, **9**, 89–94.

Hoyer,D., Engel,G. and Kalkmann,H.O. (1985) *Eur. J. Pharmacol.*, **118**, 13–23.

Hoyer,D. and Neijt,H.C. (1988) *Mol. Pharmacol.*, **33**, 303–309.

Julius,D., MacDermott,A.B., Axel,R. and Jessell,T.M. (1988) *Science*, **241**, 558–564.

Kendall,D.A. and Nahorski,S.R. (1985) *J. Pharmacol. Exp. Ther.*, **233**, 473–479.

Kobilka,B.K., Frielle,T., Collins,S., Yang-Feng,T., Kobilka,T.S., Francke,U., Lefkowitz,R.J. and Caron,M. (1987) *Nature*, **329**, 75–78.

Lefkowitz,R.J., Benovic,J.L., Kobilka,B. and Caron,M.G. (1986) *Trends Pharmacol. Sci.*, **7**, 444–448.

Leysen,J.E. (1988) In Boulten,A.A., Baker,G.B. and Juorio,A.V. (eds), *Neuromethods, Neuropharmacology* II. Humana Press, Clifton, USA, in press.

Leysen,J.E., Niemegeers,J.E., Tollenaere,J.P. and Laduron,P.M. (1978) *Nature*, **272**, 163–166.

Leysen,J.E., Awguters,F., Kennis,L., Laduron,P.M., Vandenberk,J. and Janssen,P. (1981) *Life Sci.*, **28**, 1015–1018.

Leysen,J.E., Niemegeers,J.E., Van Nueten,J.M. and Laduron,P.M. (1982) *Mol. Pharmcol.*, **21**, 301–314.

Leysen,J.E., de Courcelles,D.C., De Clerck,F., Niemegeers,J.E. and Van Nueten,J.M. (1984) *Neuropharmacology*, **23**, 1493–1501.

Lübbert,H., Hoffman,B.J., Snutch,T.P., Van Dyke,T., Levine,A.J., Hartig,P.R., Lester,H.A. and Davidson,N. (1987a) *Proc. Natl. Acad. Sci. USA*, **84**, 4332–4336.

Lübbert,H., Snutch,T.P., Pascal,N., Lester,H.A. and Davidson,D. (1987a) *J. Neurosci.*, **4**, 1159–1165.

Matsuoka,H., Ishii,M., Goto,A. and Sugimoto,T. (1985) *Am. J. Physiol.*, **234**, 234–238.

Nomura,Y., Kaneko,S., Kato,K., Yamagishi,S. and Sugiyma,H. (1987) *Mol. Brain Res.*, **2**, 113–123.

Pazos,A., Cortes,R. and Palacios,J.M. (1985) *Brain Res.*, **346**, 231–249.

Peralta,E.G., Ashkenazi,A., Winslow,J.W., Ramachandran,J. and Capon,D.J. (1988) *Nature*, **334**, 434–436.

Peralta,E.G., Askenazi,A., Winslow,J.W., Ramachandran,J. and Capon,D.J. (1988) *Nature*, **334**, 434–436.

Peroutka,S.J. (1986) *J. Neurochem.*, **47**, 529–540.

Peroutka,S.J. (1988) *Annu. Rev. Neurosci.*, **11**, 45–60.

Pritchett,D.B., Sontheimer,H., Kettenmann,H., Gorman,C., Seeburg,P.H. and Schofield,P.R. (1988) *Science*, in press.

Roth,B.L., Nakaki,T., Chuang,D.M. and Costa,E. (1984) *Neuropharmacology*, **23**, 1223–1236.

Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463–5467.

Takahashi,T., Neher,E. and Sakmann,B. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 5063–5067.

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