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# Molecular Pharmacological Differences in the Interaction of Serotonin with 5-Hydroxytryptamine$_{1C}$ and 5-Hydroxytryptamine$_2$ Receptors

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## SUMMARY

5-Hydroxytryptamine (5HT)$_{1C}$ and 5HT$_2$ receptors appear to be closely related, from a molecular viewpoint, displaying similar second messenger systems and a high degree of sequence homology. However, there are striking differences in the interactions of 5HT with 5HT$_{1C}$ and 5HT$_2$ receptors; 5HT is generally more potent in stimulating responses mediated through 5HT$_{1C}$ receptors than responses mediated through 5HT$_2$ receptors. Also [$^3$H]5HT labels 5HT$_{1C}$ receptors and not 5HT$_2$ receptors. In order to explore more fully the molecular rationale for these differences, radioligand binding studies were performed in rat, human, and porcine brain and choroid plexus tissues and in mammalian cells transfected with rat 5HT$_{1C}$ or 5HT$_2$ receptors; second messenger studies (inositol phosphate accumulation) were performed in the transfected cells. The second messenger studies confirmed the approximately 10-fold higher potency of 5HT in stimulating intracellular responses through 5HT$_{1C}$ receptors ($EC_{50}$ = 8.3 nM) than in stimulating intracellular responses through 5HT$_2$ receptors ($EC_{50}$ = 101 nM). An agonist radioligand selective for the 5HT$_{1C}$ and 5HT$_2$ receptors, 2,5-dimethoxy-(4-[$^{125}$I]iodo)phenylisopropylamine, was used, as well as [$^3$H]5HT, [$^3$H]mesulergine (antagonist radioligand for 5HT$_{1C}$ receptors), and [$^3$H]ketanserin (antagonist radioligand for 5HT$_2$ receptors). Computer-assisted analyses of the binding data revealed two agonist affinity states for the 5HT$_{1C}$ receptor. The agonist high affinity state of the receptor was modifiable by guanyl nucleotides. The proportion of agonist high affinity states, relative to the total receptor population, was approximately 10% for both receptors. The apparent higher affinity of 5HT for the radiolabeled 5HT$_{1C}$ receptors was due to the higher affinity 5HT displayed for the agonist low affinity state of the 5HT$_{1C}$ receptor, compared with the affinity of 5HT for the agonist low affinity state of the 5HT$_2$ receptor. The correspondence between the higher affinity of 5HT for the agonist low affinity state of the 5HT$_{1C}$ receptor, relative to the 5HT$_2$ receptor, and the higher potency of 5HT in stimulating 5HT$_{1C}$ responses indicates that 5HT interacts with the agonist low affinity state of the 5HT$_{1C}$ and 5HT$_2$ receptors in initiating its biological effects. These observations indicate that guanine nucleotide-binding protein (G protein)-coupled receptors can exhibit high affinity for neurotransmitters in both the free receptor and the G protein-coupled states and that receptors exhibiting this property may represent a novel subfamily of G protein-coupled receptors.

Since the first description of two different tryptamine receptors in 1957 (1), several new serotonin (5HT) receptor subtypes have been reported. To date, seven distinct serotonin receptors have been identified, and evidence for more exists (2, 3). Serotonin receptors belong to two different receptor superfamilies, the G protein-coupled receptors and the ligand-gated ion channels. The 5HT$_1$ and 5HT$_2$ receptors belong to the former and the 5HT$_3$ receptors to the latter superfamily (4, 5). The 5HT$_1$ receptor family, originally identified by its high affinity for [$^3$H]5HT (6), has been divided into several subtypes, called 5HT$_{1A}$, 5HT$_{1B}$ (7), 5HT$_{1C}$ (8, 9), and 5HT$_{1D}$ (10). Recently, it has been suggested that the 5HT$_{1C}$ receptor be considered as a subtype of the 5HT$_2$ receptor family, based on similarities in molecular structure and pharmacological properties (4).

The 5HT$_{1C}$ and the 5HT$_2$ receptors share similar characteristics. Both receptors are coupled to G proteins and phosphoinositol metabolism (11) and share similar pharmacological profiles [e.g., hallucinogenic phenylisopropylamines have high affinities for both receptors (12)]. Furthermore, the amino acid sequence and, presumably, the three-dimensional structures of the 5HT$_{1C}$ and the 5HT$_2$ receptors are very similar. Overall, the 5HT$_{1C}$ and 5HT$_2$ receptors exhibit 51% sequence homology, which is greatest in the transmembrane domains (~80%) (13,

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[1] Formerly spelled Titeler.

**ABBREVIATIONS:** 5HT, 5-hydroxytryptamine; DOB, 2,5-dimethoxy-(4-bromo)phenylisopropylamine; DOI, 2,5-dimethoxy-(4-iodo)phenylisopropylamine; G protein, guanine nucleotide-binding protein; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; Gpp(NH)p, guanosine-5′-(2,3-imido)triphosphate; LSD, lysergic acid diethylamide; TFMPP, *m*-trifluoromethylphenylpiperazine; DOM, 4-methyl(2,5-dimethoxy)phenylisopropylamine; MDA, 3,4-methylenedioxyamphetamine.

14). The most striking difference between these two receptor subtypes appears to be the affinity of the endogenous ligand serotonin. Because the $5HT_{1C}$ and the $5HT_2$ receptors belong to the superfamily of G protein-coupled receptors, the agonist serotonin should have nanomolar affinity for both agonist-radiolabeled receptors (agonist high affinity states) and micromolar affinity for the antagonist-radiolabeled $5HT_{1C}$ and $5HT_2$ receptors (agonist high and low affinity states). However, 5HT has nanomolar affinity for the antagonist-radiolabeled $5HT_{1C}$ receptor (9, 15, 16) and micromolar affinity for the antagonist-radiolabeled $5HT_2$ receptor (17, 18). Tritiated serotonin labels the $5HT_{1C}$ receptor, as determined in homogenate binding assays and by autoradiography in brain tissue (8, 19). Attempts to label the $5HT_2$ receptor with [$^3$H]5HT in brain tissue have not been successful, presumably due to the low density of agonist high affinity states. Other tritiated and iodinated agonists with higher specific activity than [$^3$H]5HT, i.e., [$^3$H]DOB (17) and [$^{125}$I]DOI (20), can label the agonist high affinity state of the $5HT_2$ receptor.

In the present study, we attempted to answer the question of why 5HT displays high affinity for the antagonist-labeled $5HT_{1C}$ receptor and to explain why [$^3$H]5HT labels the $5HT_{1C}$ receptor but not the $5HT_2$ receptor. We report here that the agonist high (receptor-G protein complex) and the agonist low (free receptor) affinity states for the $5HT_{1C}$ receptor show relatively high affinity for 5HT. Therefore, we propose that the molecular rationale for the higher overall affinity of 5HT for the $5HT_{1C}$ receptor is based on the higher affinity of 5HT for the free $5HT_{1C}$ receptor (agonist low affinity state). We further propose that this higher overall affinity of 5HT for the agonist low affinity state of the $5HT_{1C}$ receptor is also responsible for the higher potency of 5HT for $5HT_{1C}$ receptor-mediated responses. This higher overall affinity of 5HT for the free $5HT_{1C}$ receptor is in contrast to most G protein-coupled receptors, including the $5HT_2$ receptor (21). Generally, the endogenous neurotransmitters exhibit a much lower affinity for the free receptor than serotonin appears to display for the free (uncoupled) $5HT_{1C}$ receptor.

## Materials and Methods

Stably transfected cell lines expressing the $5HT_{1C}$ receptor and $5HT_2$ receptor were generously donated by Dr. David Julius (Department of Pharmacology, University of California San Francisco) (22) or produced by Dr. Beth Hoffman as previously described (23). The cells were grown in Dulbecco's modified Eagle's medium with L-glutamine (2 mM/mL), penicillin/streptomycin (200 units/mL), G418, and fetal bovine serum (10%), in culture flasks, until confluency. Cells were harvested in 50 mM Tris·HCl buffer, with 0.5 mM EDTA and 10 mM $MgSO_4$, and were centrifuged at 7000 × *g* for 30 min. The supernatant was discarded, and the pellet was resuspended in buffer and incubated at 37° for 15 min. After an additional centrifugation for 20 min at 7000 × *g*, the pellets were stored at −20° until use. Post-mortem human frontal cortex was obtained from the Albany Medical Center Pathology Department. Human tissue was obtained at autopsy from one male subject, age 28 (post-mortem delay, 8 hr), who had no history of neurological or psychiatric disease and was not known to be taking any psychoactive medication. Neuropathological examination of the tissues verified the absence of neurological disease. Pig brains were purchased fresh at Veteran's Pork (Veteran, NY). Rats were purchased from Taconic Farms and killed by decapitation, and brains were immediately placed on ice. The brains were dissected over ice. Tissues were homogenized (Kinematica Polytron) in cold buffer and centrifuged at 30,000 × *g* for 15 min. The buffer was identical for all tissue and cell membrane preparations. The pellets were resuspended in buffer, incubated at 37° for 15 min, and recentrifuged twice, for 10 min each time, with a resuspension between centrifugations. The pellets were stored at −20° until use.

Radioligand binding studies were performed in triplicate, in a final volume of 2 ml containing 0.1 ml of radioligand, 0.1 ml of buffer or competing drug, 0.8 ml of buffer (containing 0.1% ascorbate and 10 μM pargyline), and 1 ml of membranes. Tris·HCl-based buffer with 0.1% ascorbate and 10 μM pargyline has been found to eliminate the oxidation and degradation of 5HT, which can interfere with radioligand binding assays (24). Saturation experiments for the $5HT_{1C}$ receptor were performed using increasing concentrations of [$^{125}$I]DOI, [$^3$H]5HT, and [$^3$H]mesulergine, in the presence and absence of 10 μM mianserin. Saturation experiments for the $5HT_2$ receptor were performed using increasing concentrations of [$^{125}$I]DOI and [$^3$H]ketanserin, in the presence and absence of 10 μM ketanserin. Saturation studies were performed using $^{125}$I-DOI concentrations ranging from 3 to 0.03 nM, [$^3$H]mesulergine concentrations ranging from 10 to 0.03 nM, [$^3$H]5HT concentrations ranging from 40 to 0.3 nM, and [$^3$H]ketanserin concentrations ranging from 10 to 0.3 nM. Competition experiments were performed using 0.05 nM [$^{125}$I]DOI, 1.0 nM [$^3$H]mesulergine, 0.4 nM [$^3$H]-ketanserin, and 0.6 and 20 nM [$^3$H]5HT. At these concentrations, 70–100% of the binding was specific. Ten concentrations of competing drugs were used, ranging from 0.35 nM to 10 μM. At the highest concentration of competing drugs, 90–100% of the specific binding was inhibited. Assay tubes were incubated at 22° for 30 min, filtered on Schleicher & Schuell glass fiber filters (presoaked in 0.1% polyethyleneimine), and washed with 10 ml of ice-cold buffer. Filters from tritiated competition assays were incubated for 3 hr in vials containing 5 ml of aqueous scintillant (Ecoscint; National Diagnostic) and were counted on a Beckman 3801 liquid scintillation counter, at an efficiency of 50%. Iodinated filters were counted on a LKB-Wallac γ counter, at 80% efficiency. Saturation and competition experiments were analyzed using EBDA (25) and RS1 (BBN Software) programs, to obtain equilibrium dissociation constants ($K_d$), kinetic constants, $B_{max}$ values, Hill coefficients, and $IC_{50}$ values. Whenever curves were modeled best by a one-site fit, $K_i$ values were derived from $IC_{50}$ values by using the Cheng-Prusoff equation, $K_i = IC_{50}/[1 + (D/K_d)]$, where $D$ is the level of radioligand and $K_d$ is the equilibrium dissociation constant of the radioligand for the receptor (26). Graphs were obtained using SigmaPlot (Jandel Scientific). Proteins were determined by the Pierce bicinchoninic acid assay. To measure inositol phosphate accumulation, $1 \times 10^5$ cells/well were plated into 12-well plates and incubated overnight in medium supplemented with 2.5% fetal calf serum and 2.5 μCi of *myo*-[$^3$H]inositol (American Radiolabeled Chemicals, Inc., St. Louis, MO). After incubation, the cells were washed twice with medium containing 10 mM LiCl and were incubated for 15 min with serotonin. Reactions were stopped with stop solution, containing 1 M KOH, 18 mM sodium borate, 3.8 mM EDTA, and 7.6 mM NaOH, and were neutralized with 1 volume of 7.5% HCl. Elution of inositol phosphates was performed according to the method of Berridge *et al.* (27).

The radioligands [$^{125}$I]DOI (2200 Ci/mmol), [$^3$H]5HT (23.4 Ci/mmol), and [$^3$H]ketanserin (75.9 Ci/mmol) were purchased from New England Nuclear. [$^3$H]Mesulergine (73 Ci/mmol) was purchased from Amersham. Guanyl nucleotides were obtained from Boehringer. Competing drugs were purchased from Research Biochemicals Incorporated or from Sigma. Buffer components were obtained either from Sigma or from Fisher. Cell growth media were purchased from GIBCO-BRL.

## Results

In order to compare potencies of 5HT in stimulating $5HT_{1C}$ receptor-mediated and $5HT_2$ receptor-mediated events in transfected mammalian cells and mammalian brain tissue, 5HT-stimulated inositol phosphate production in transfected fibroblasts was measured. The $EC_{50}$ values for 5HT with J1 cells expressing $5HT_{1C}$ and GF6 cells expressing $5HT_2$ receptors were $EC_{50} = 8.3 \pm 0.6$ nM for $5HT_{1C}$ and $EC_{50} = 101 \pm 15$ nM for $5HT_2$ (Table 1). The 10-fold higher potency of 5HT for the

TABLE 1

**Potency of serotonin in stimulating $5HT_{1C}$ and $5HT_2$ receptor-mediated phosphatidylinositol turnover in mammalian brain and stably transfected fibroblasts**

Apparent $EC_{50}$ values for serotonin-stimulated phosphatidylinositol turnover were measured in rat cerebral cortical slices and GF6 cells ($5HT_2$ receptor mediated) and in rat choroid plexus and J1 cells ($5HT_{1C}$ receptor mediated).

| | $EC_{50}$ | |
|---|---|---|
| | $5HT_{1C}$ receptor-mediated | $5HT_2$ receptor-mediated |
| | nM | |
| Transfected mammalian cell line | 8.3 ± 0.6 | 101 ± 15 |
| Mammalian brain tissue | 46[a] | 540[a] |

[a] From Conn et al. (28).

$5HT_{1C}$ receptor-mediated responses correlates well with previously published results of Conn *et al.* (28), in mammalian brain tissue, indicating that 5HT is more potent in stimulating $5HT_{1C}$ receptor-mediated responses then in stimulating $5HT_2$ receptor-mediated responses.

As shown in Table 2, specific [$^3$H]mesulergine, specific [$^3$H]5HT, and specific [$^{125}$I]DOI binding to $5HT_{1C}$ receptors demonstrated high affinity in pig choroid plexus and cell membrane homogenates. In pig choroid plexus, 14% of the total receptor number was labeled by the agonist ligand [$^{125}$I]DOI. Nine percent of the antagonist-radiolabeled $5HT_{1C}$ receptors in J1 cells were labeled with [$^{125}$I]DOI. Fig. 1 displays the saturable specific binding of [$^3$H]5HT to $5HT_{1C}$ receptors in J1 membrane homogenates. The biphasic Scatchard analysis of the saturation data (Fig. 1, *inset*) indicates that two sites or two affinity states were being recognized by the agonist radioligand ($p = 0.02$ for a two-site fit). The high affinity site, which comprised 25% of the receptors, had a $K_d$ of 0.3 nM; the remaining site had a $K_d$ of 7 nM. The estimated $B_{max}$ for [$^3$H]5HT-labeled $5HT_{1C}$ receptors was 6440 fmol/mg of protein. The Scatchard analysis of saturable specific [$^3$H]5HT binding in pig choroid plexus was best fit by a single-site analysis and indicated a $B_{max}$ of 739 ± 150 fmol/mg of protein and a $K_d$ of 4.03 ± 1.1 nM (Table 2). The pharmacological characteristics of specific [$^3$H]mesulergine and [$^{125}$I]DOI binding to brain and transfected cell $5HT_{1C}$ receptors are shown in Table 3. The rank order of affinities of various agonists and antagonists for [$^3$H]mesulergine-labeled $5HT_{1C}$ receptors corresponds well to previously reported $5HT_{1C}$ receptor characteristics (29). Serotonin showed highest affinity for the $5HT_{1C}$ receptor in both tissues, followed by (±)-DOB > (±)-DOI > (±)-DOM > (−)-MDA. Among the antagonists, mianserin and mesulergine had highest affinity for the $5HT_{1C}$ receptor, followed by ketanserin > cinanserin. Spiperone had a characteristically low affinity for the $5HT_{1C}$ receptor in pig choroid plexus and J1 membrane homogenates. For [$^{125}$I]DOI-labeled $5HT_{1C}$ receptors, the rank order of affinities was identical to that of the [$^3$H]mesulergine-labeled receptors. In both tissues, the absolute affinities of the competing agonists were higher for the [$^{125}$I]-DOI-labeled $5HT_{1C}$ receptor than for the [$^3$H]mesulergine-radiolabeled $5HT_{1C}$ receptor. All drugs displaced 90–100% of the specific binding determined with $10^{-5}$ M mianserin. No specific binding could be detected in untransfected cell membrane homogenates.

A working hypothesis was developed, that low concentrations (0.1–1 nM) of [$^3$H]5HT should label the agonist high affinity state of the $5HT_{1C}$ receptor and that higher concentrations of [$^3$H]5HT should label the agonist low affinity state of the $5HT_{1C}$ receptor. In order to investigate the effect of different concentrations of [$^3$H]5HT on $5HT_{1C}$ receptor competition studies, we measured serotonin, (±)-DOI, mesulergine, and ketanserin competition for 0.6 nM and 20 nM [$^3$H]5HT binding, as shown in Table 4 and Fig. 2. The $IC_{50}$ values for the antagonists mesulergine and ketanserin did not change significantly, regardless of the concentration of radioligand used. There was no significant change in Hill coefficients at either concentration of radioligand. In contrast to the antagonist competition studies, agonists had a higher affinity for the 0.6 nM [$^3$H]5HT-labeled $5HT_{1C}$ receptor than for the 20 nM [$^3$H]5HT-radiolabeled receptor. (±)-DOI competition with high and low concentrations of [$^3$H]5HT caused an approximately 12-fold shift in the affinity of (±)-DOI for the $5HT_{1C}$ receptor. The shift in the serotonin competitions was somewhat less (approximately 8-fold), presumably because serotonin has a higher affinity than (±)-DOI for the free $5HT_{1C}$ receptor.

It has been shown that addition of guanyl nucleotides to agonist competition studies with antagonist-radiolabeled $5HT_2$ receptors causes a rightward shift in the competition curve, due to the conversion of agonist high affinity states (receptor-G protein complex) to agonist low affinity states (free receptor) (30). Fig. 3 shows a representative competition curve for the agonist DOB with [$^3$H]mesulergine-labeled $5HT_{1C}$ receptors in pig choroid plexus and transfected cell membranes, in the presence and absence of 10 μM Gpp(NH)p. The addition of 10 μM Gpp(NH)p changed the $IC_{50}$ value from 14.5 nM to 29 nM in pig choroid plexus and from 68 nM to 127 nM in J1 cell membranes. Hill coefficients were 0.45 and 0.63 in the absence and presence, respectively, of the guanyl nucleotide in pig choroid. In J1 cell membranes the Hill coefficient shifted from 0.73 to 0.93 in the presence of Gpp(NH)p.

In order to compare the pharmacological properties of the $5HT_{1C}$ receptor and the $5HT_2$ receptor expressed in transfected mammalian cells, we studied the $5HT_2$ receptor expressed in NIH-3T3 cells. Saturation experiments and competition assays in brain tissue were also performed, for comparison. The density of [$^3$H]ketanserin-labeled $5HT_2$ sites in rat and human frontal cortex, as shown in Table 5, was similar to previously published results (31). In GF6 cell membranes, Scatchard analysis of [$^3$H]ketanserin saturation data indicated a $B_{max}$ of

TABLE 2

**Receptor densities and dissociation constants for the $5HT_{1C}$ receptor in mammalian brain tissue and J1 cells**

Values are derived from Scatchard analysis of saturation experiments. Six different concentrations of radioactive drug were used for three independent saturation experiments performed in triplicate. Mianserin ($10^{-6}$ M) was used to define nonspecific binding.

| Receptor | [$^3$H]Mesulergine | | [$^{125}$I]DOI | | [$^3$H]5HT | |
|---|---|---|---|---|---|---|
| | $B_{max}$ | $K_D$ | $B_{max}$ | $K_D$ | $B_{max}$ | $K_D$ |
| | fmol/mg | nM | fmol/mg | nM | fmol/mg | nM |
| 5-$HT_{1C}$, pig choroid | 688 ± 34 | 4.6 ± 0.8 | 98 ± 6 | 1.6 ± 0.4 | 739 ± 150 | 4.03 ± 1.1 |
| 5-$HT_{1C}$, J1 cells | 4750 ± 900 | 4 ± 0.6 | 430 ± 50 | 4.8 ± 0.1 | 6440 ± 850 | $K_{D_{high}}$, 0.3; $K_{D_{low}}$, 7.0 |

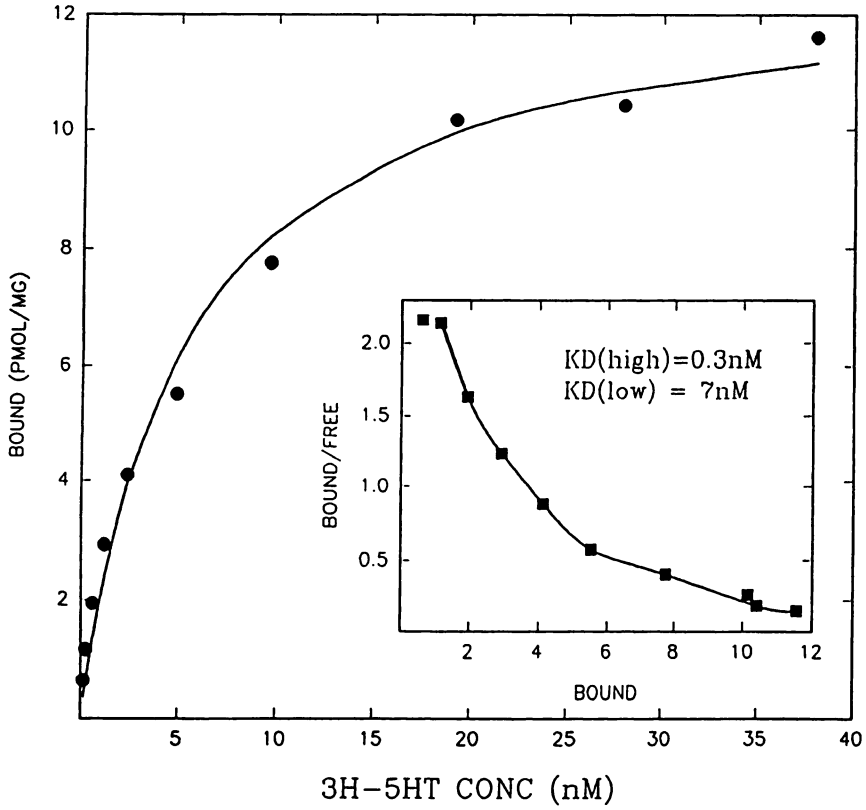


**Fig. 1.** Saturation data and Scatchard analysis (inset) for [$^3$H]5HT-labeled 5HT$_{1C}$ receptors in transfected mammalian cell membrane homogenates. The biphasic Scatchard analysis ($p$ = 0.02 for a two-site fit) indicates that two receptor sites or affinity states are being recognized by the agonist ligand. Mianserin ($10^{-5}$ M) was used to define nonspecific binding. Data points are the result of three independent experiments performed in triplicate. Standard errors are <5% for each point.

TABLE 3

**Competition experiments for [$^{125}$I]DOI- and [$^3$H]mesulergine-labeled 5-HT$_{1C}$ receptors in pig choroid plexus and J1 cells**

Reported values are the mean ± standard error for three independent experiments performed in triplicate. Ten concentrations of competing drug were used.

| | $K_i$ | | | |
|---|---|---|---|---|
| | [$^3$H]Mesulergine | | [$^{125}$I]DOI | |
| | Choroid plexus | J1 cells | Choroid plexus | J1 cells |
| | nM | | | |
| Agonist | | | | |
| 5HT | 11 ± 1 | 23 ± 2 | 2 ± 1 | 0.7 ± 0 |
| (±)-DOB | 20 ± 4 | 60 ± 2 | 4 ± 0.5 | 3 ± 0.1 |
| (±)-DOI | 35 ± 4 | 45 ± 8 | 2 ± 0.2 | 3 ± 1 |
| (±)-DOM | 86 ± 3 | 370 ± 24 | 7 ± 0.2 | 41 ± 8 |
| (−)-MDA | 450 ± 68 | 2740 ± 219 | 48 ± 1 | 118 ± 5 |
| Antagonist | | | | |
| Mesulergine | 2 ± 0.3 | 3 ± 0.1 | 4 ± 1 | 2 ± 0.3 |
| Mianserin | 2 ± 0.4 | 2 ± 0.1 | 3 ± 0.4 | 2 ± 0.4 |
| Ketanserin | 35 ± 4 | 21 ± 4 | 130 ± 17 | 47 ± 9 |
| Cinanserin | 73 ± 0.3 | | 178 ± 13 | |
| Spiperone | 735 ± 601 | 1041 ± 211 | 1989 ± 88 | 2114 ± 211 |

TABLE 4

**Competition experiments for [$^3$H]5HT-labeled 5-HT$_{1C}$ receptors in J1 cell membranes**

Reported IC$_{50}$ values are the mean ± standard error for three to five independent experiments performed in triplicate. Ten concentrations of competing drug were used.

| Competing drug | 0.6 nM [$^3$H]5HT | | 20 nM [$^3$H]5HT | |
|---|---|---|---|---|
| | IC$_{50}$ | Hill coefficient | IC$_{50}$ | Hill coefficient |
| | nM | | nM | |
| 5HT | 4 ± 0.4 | 0.8 ± 0.03 | 31 ± 0.9 | 0.9 ± 0.02 |
| (±)-DOI | 5 ± 0.5 | 0.8 ± 0.03 | 63 ± 6 | 0.9 ± 0.03 |
| Mesulergine | 3 ± 0.4 | 1.1 ± 0.05 | 7 ± 0.4 | 0.8 ± 0.04 |
| Ketanserin | 50 ± 3 | 0.8 ± 0.03 | 130 ± 11 | 0.7 ± 0.03 |

7700 fmol/mg. The agonist radioligand [$^{125}$I]DOI labeled 7.5%, 5.5%, and 19.4% of the total receptor amount labeled with the antagonist radioligand in rat, human, and GF6 cells, respectively. For both the 5HT$_{1C}$ and the 5HT$_2$ receptor, [$^{125}$I]DOI labeled a fraction of the total receptor population labeled by the antagonist radioligand. Table 6 shows the $K_i$ values for competition studies of various agonists and antagonists competing for [$^{125}$I]DOI- and [$^3$H]ketanserin-labeled 5HT$_2$ receptors. In GF6 cells and rat frontal cortex, the rank order of affinities of the competing agonists for the [$^{125}$I]DOI-labeled 5HT$_2$ receptors was (±)-DOB > 5HT > LSD > TFMPP > tryptamine. The rank order of affinities of antagonists under the same conditions was spiperone > ketanserin > chlorpromazine (> cinanserin). In human tissue, the rank order of agonists for the agonist-radiolabeled receptors varied somewhat, with LSD being the most potent competing drug, followed by (±)-DOB > 5HT > TFMPP > tryptamine. When [$^3$H]ketanserin was used to radiolabel the 5HT$_2$ receptor, the rank order of agonist $K_i$ values in rat frontal cortex and GF6 cells was: LSD > (±)-DOB > TFMPP > 5HT > tryptamine. Antagonists compete with the following rank order: spiperone > ketanserin > chlorpromazine (> cinanserin). There was a statistically significant difference in affinity for spiperone between rat and human cortical tissues; this may be due to subtle differences in the receptor itself or may be due to some contaminant in the membrane homogenate. Nonhydrolyzable analogs of GTP potently inhibited 57–61% of specific [$^{125}$I]DOI binding to 5HT$_2$ receptors in both tissues. Similar to the 5HT$_2$ receptor, 56–61% of specific [$^{125}$I]DOI binding to the 5HT$_{1C}$ receptor could be inhibited by $10^{-5}$ M guanyl nucleotides (Table 7).

## Discussion

Agonist and antagonist radioligands have been used in radioligand binding studies to label many G protein-coupled recep-

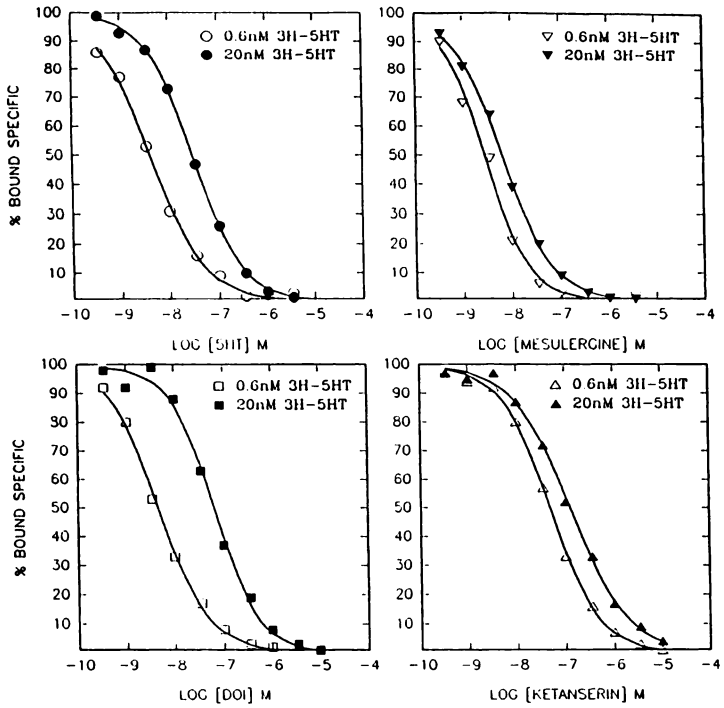


**Fig. 2.** Agonist and antagonist competition curves for [$^3$H]5HT-labeled $5HT_{1C}$ receptors in J1 cell membrane homogenates. Two concentrations of radioligand were used, and the assays were run in parallel. Data points are the result of three independent experiments performed in triplicate. Standard errors are <5% for each data point. The small shift in the $IC_{50}$ values for antagonist competition can be explained by the Cheng-Prusoff equation.

tors (32). These studies have revealed that G protein-coupled receptors exist in two interconvertible states, the receptor-G protein complex and the free receptor. The two receptor states differ in their affinity for agonist radioligands, with agonists having higher affinity for the receptor-G protein complex. Antagonist radioligands are thought to interact with both states, the receptor-G protein complex (agonist high affinity state) and the free receptor (agonist low affinity state), with equal affinity. Radioligand binding studies of the $5HT_2$ receptor have been consistent with the hypothesis that this serotonin receptor exists in two agonist affinity states (23, 31, 33). In contrast to the $5HT_2$ receptor, radioligand binding studies of the $5HT_{1C}$ receptor in pig choroid plexus have shown that [$^3$H] 5HT labels the same number of receptors as the antagonist [$^3$H]mesulergine (8). This is not consistent with the classic ternary complex theory (34). Second messenger studies assessing the potency of 5HT in $5HT_{1C}$ and $5HT_2$ receptor-mediated phosphoinositol turnover demonstrated that serotonin is >10-fold more potent at $5HT_{1C}$ receptors than at $5HT_2$ receptors (35). The recent advances in molecular biology techniques, i.e., the cloning of the $5HT_{1C}$ and $5HT_2$ receptor genes and their expression in stably transfected mammalian cell lines, presented the opportunity to investigate further the pharmacological and molecular differences between the $5HT_{1C}$ and $5HT_2$ receptors.

The pharmacological properties of $5HT_{1C}$ and $5HT_2$ receptors expressed in transfected mammalian cell lines appear to be very similar to those of native receptors expressed in brain tissue. The transfected cells exhibited a similar higher sensitivity (potency) to stimulation by 5HT when transfected with the $5HT_{1C}$ receptor than when transfected with the $5HT_2$ receptor; this is consistent with studies performed in heterogeneous tissues such as brain tissue slices. There is a possibility that the observed potency difference may be due to an increased spare receptor population in the $5HT_{1C}$ cells; this possibility was investigated in brain tissue and found to be a minor factor in 5HT potency (36). Studies to determine the spare receptor situation in the recombinant cells are currently being planned. Interestingly, Scatchard analysis of the saturation experiments on transfected cell line-derived $5HT_{1C}$ receptors with the agonist [$^3$H]5HT was best fit by a two-site analysis. Because the mammalian cell line was transfected with a single cDNA coding for a single receptor, we hypothesized that [$^3$H]serotonin labels the receptor-G protein complex and the free receptor. Traditionally, agonist radioligands were thought to label only the receptor-G protein complex, due to their very low affinity for the free receptor (0.7–2 $\mu$M range) (21, 37). However, in the case of [$^3$H]5HT binding to the $5HT_{1C}$ receptor of transfected mammalian cell membranes, the agonist radioligand appears to label also the agonist low affinity state. When the density of high affinity [$^3$H]5HT sites in J1 cells is compared with the number of sites labeled by [$^{125}$I]DOI, it is apparent that [$^3$H] 5HT appears to label twice the number of high affinity sites. A possible explanation for this discrepancy is that computer-assisted analyses produce estimations of percentages of high and low affinity states; the actual levels of high affinity states labeled by [$^3$H]5HT might be lower than estimated by the computer analysis. The [$^{125}$I]DOI saturation curve for the $5HT_{1C}$ receptor was best fit by a one-site model, as opposed to the [$^3$H]5HT saturation curve, which was best fit by a two-site model; this is undoubtedly due to the higher affinity for 5HT displayed by the low affinity state (free receptor) of the $5HT_{1C}$ receptor. At the maximal concentrations of [$^{125}$I]DOI used, low fractional occupancy of the low affinity state of the $5HT_{1C}$ receptor would be anticipated. The main point is that agonist radioligands are labeling a high affinity state that represents a minority of the total receptor population.

To substantiate further the finding that the agonist high and low affinity states were being labeled, we investigated the effect of high and low concentrations of [$^3$H]5HT in agonist and antagonist competition studies. Agonists and antagonists competed differently for the high and low radioligand concentra-

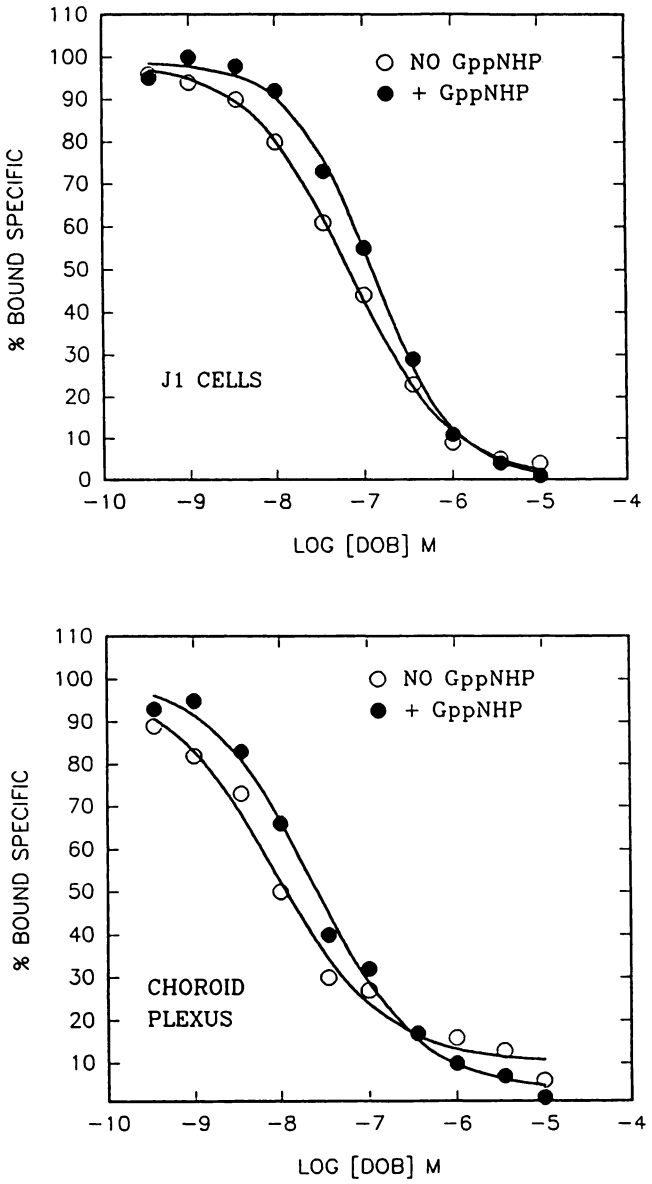


**Fig. 3.** Representative agonist competition curves for [$^3$H]mesulergine-labeled $5HT_{1C}$ receptors in J1 cell membranes and pig choroid plexus, in the presence and absence of $10^{-5}$ M Gpp(NH)p. The rightward shift of the competition curve in the presence of Gpp(NH)p is typical for G protein-coupled receptors.

TABLE 5

**Receptor densities and dissociation constants for the $5HT_2$ receptor in mammalian brain tissue and stably transfected GF6 cells**

Values are derived from Scatchard analysis of saturation experiments. Six different concentrations of radioactive drug were used for three independent saturation experiments performed in triplicate. Cinanserin ($10^{-5}$ M) was used to define non-specific binding.

| Receptor | [$^3$H]Ketanserin | | [$^{125}$I]DOI | |
|---|---|---|---|---|
| | $B_{max}$ | $K_d$ | $B_{max}$ | $K_d$ |
| | fmol/mg | nM | fmol/mg | nM |
| $5HT_2$, rat cortex | 358 ± 8 | 0.6 ± 0.03 | 27 ± 5 | 0.4 ± 0.08 |
| $5HT_2$, human cortex | 283 ± 9 | 1.1 ± 0.06 | 15.8 ± 3 | 0.2 ± 0.05 |
| $5HT_2$, GF6 cells | 7700 | 0.8 ± 0 | 1500 | 0.8 ± 0.1 |

tions. Raising the radioligand concentration caused a rightward shift of the 5HT and DOI competition curves and an increase in the $IC_{50}$ values. The higher radioligand concentrations caused a minimal shift (approximately 2-fold) in the antagonist $IC_{50}$ values. In the case of the agonist competition studies against 0.6 and 20 nM [$^3$H]5HT, the significant change in the $IC_{50}$ values (approximately 8- and 12-fold for 5HT and DOI, respectively) indicates multiple affinity states of the receptor. If, at higher concentrations, [$^3$H]5HT labels the receptor-G protein complex and the free receptor, competing agonists also compete for the agonist low affinity state (free receptor). This agonist competition for the [$^3$H]5HT-labeled agonist low affinity state is then responsible for the higher $IC_{50}$ values. The Hill coefficients for all the drugs ranged between 0.8 and 1.1, with the exception of ketanserin, which displayed a Hill coefficient of 0.7. The differences between affinities of the two states of the receptor for the drugs would not be expected to produce dramatic lowering of Hill coefficients. The Hill coefficient for ketanserin was statistically less than unity; there may be a larger difference in affinities between the two states of the receptor for ketanserin than for the other drugs.

Our hypothesis that [$^3$H]5HT labels the free $5HT_{1C}$ receptor is further supported by the small shift in agonist competition curves upon addition of Gpp(NH)p. Addition of guanyl nucleotides to an agonist competition assay with antagonist-radiolabeled G protein-coupled receptors typically causes a rightward shift of the competition curves and an increase in the $K_i$ values. The magnitude of the shift is dependent on the relative amount of receptors forming receptor-G protein complexes. Because normally <15% of the total receptor number are thought to be in the G protein-coupled state, addition of guanyl nucleotides causes only small but important changes in the $K_i$ value and in the shape of the curve. The small changes in the shape of the curve and the $K_i$ for an agonist (DOB) in competition for antagonist-radiolabeled $5HT_{1C}$ receptors are presumably based on the fact that only 9 and 14% of the receptors in the transfected cells and in pig choroid plexus, respectively, are coupled to G proteins. Therefore, the data support our hypothesis that [$^3$H]5HT labels the free $5HT_{1C}$ receptor, because addition of guanyl nucleotides in agonist competition assays confirms that only a fraction of the $5HT_{1C}$ receptors exist in the agonist high affinity state. In the mammalian cell line transfected with rat $5HT_2$ receptor cDNA, about 20% of the total receptor number appears to be in the G protein-coupled state and is labeled with [$^{125}$I]DOI. The amount of $5HT_2$ receptors labeled by [$^{125}$I]DOI is dependent on the amount of receptor-G protein coupling in the cell membranes and the concentration of G proteins. Such a concentration might be cell type dependent and would explain the relatively high percentage of specific [$^{125}$I]DOI binding in the GF6 cells. In all tissues and cell membranes examined, specific [$^{125}$I]DOI binding to the $5HT_{1C}$ and $5HT_2$ receptors could be inhibited by addition of 10 μM guanyl nucleotides, as was previously shown for [$^3$H]DOB-labeled $5HT_2$ receptors (17).

In summary, the radioligand binding studies reported herein are supportive of classical ternary complex (G protein-coupled) receptor radioligand binding properties for the $5HT_2$ receptor and show unusual radioligand binding properties for the $5HT_{1C}$ receptor. We propose that the free, G protein-uncoupled, state of the $5HT_{1C}$ receptor exhibits sufficiently high affinity for 5HT to allow direct radiolabeling with [$^3$H]5HT and to explain

TABLE 6

**Competition experiments for [$^{125}$I]DOI and [$^{3}$H]ketanserin-labeled $5HT_2$ receptors in transfected cells and rat and human frontal cortex**

Reported values are the mean ± standard error of three independent experiments performed in triplicate. Ten or 11 concentrations of competing drug were used. Nonspecific binding was determined with $10^{-5}$ M spiperone or ketanserin.

| $5HT_2$ receptor | $K_i$ | | | | |
|---|---|---|---|---|---|
| | [$^{3}$H]Ketanserin | | [$^{125}$I]DOI | | |
| | GF6 cells | Rat frontal cortex | GF6 cells | Rat frontal cortex | Human cortex |
| | nM | | | | |
| Agonists | | | | | |
| LSD | 12 ± 1 | 1.3 ± 0.3 | 7 ± 0.3 | 1.3 ± 0.1 | 1.1 ± 0.2 |
| (±)-DOB | 85 ± 4 | 146 ± 9 | 1 ± 0.3 | 1 ± 0.01 | 2 ± 0.3 |
| 5HT | 510 ± 30 | 928 ± 67 | 2 ± 0.4 | 1 ± 0.4 | 7 ± 0.6 |
| TFMPP | 300 ± 70 | 161 ± 4 | 12 ± 4 | 6 ± 0.4 | 30 ± 2 |
| Tryptamine | 3920 ± 368 | 2005 ± 116 | 25 ± 9 | 8 ± 2 | 69 ± 26 |
| Antagonists | | | | | |
| Ketanserin | 1 ± 0.1 | 1 ± 0.04 | 3 ± 0.1 | 1 ± 0.04 | 2 ± 0.2 |
| Spiperone | 1 ± 0.06 | 0.8 ± 0.04 | 2 ± 0.1 | 0.4 ± 0.04 | 3 ± 0.4 |
| Cinanserin | Not done | 7 ± 2 | Not done | 5 ± 0.03 | 10 ± 2 |
| Chlorpromazine | 3 ± 0.4 | 4 ± 0.8 | 8 ± 1 | 3 ± 0.03 | 13 ± 2 |

TABLE 7

**Inhibition of specific [$^{125}$I]DOI binding to $5HT_{1C}$ and $5HT_2$ receptors by guanyl nucleotides in brain and transfected cell lines**

Ten concentrations of guanyl nucleotide competed for [$^{125}$I]DOI-labeled $5HT_{1C}$ and $5HT_2$ receptors. Adenyl nucleotides at $10^{-5}$ M did not inhibit specific [$^{125}$I]DOI binding to the serotonin receptors in brain tissue and transfected cell membranes.

| Receptor | $IC_{50}$ | | |
|---|---|---|---|
| | Gpp(NH)p, brain | Gpp(NH)p, cells | GTPγS, cells |
| | nM | | |
| $5HT_{1C}$ | 23 ± 7.1 (pig choroid) | 22 ± 2 (J1 cells) | 4 ± 0.5 (J1 cells) |
| $5HT_2$ | 88 ± 27 (human cortex) | 7 ± 5 (GF6 cells) | 4 ± 1.5 (GF6 cells) |

the higher potency of 5HT in stimulating responses evoked through the $5HT_{1C}$ receptor, relative to the $5HT_2$ receptor.

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