Pharmacology Biochemistry & Behavior, Vol. 35, pp. 211–217. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

[^{125}I]-2-(2,5-Dimethoxy-4-Iodophenyl)Aminoethane ([^{125}I]-2C-I) as a Label for the 5-HT$_2$ Receptor in Rat Frontal Cortex

MICHAEL P. JOHNSON, CHESTER A. MATHIS,[1] ALEXANDER T. SHULGIN,[1] ANDREW J. HOFFMAN AND DAVID E. NICHOLS[2]

Departments of Medicinal Chemistry and Pharmacognosy, and Pharmacology and Toxicology School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907

Received 5 July 1989

JOHNSON, M. P., C. A. MATHIS, A. T. SHULGIN, A. J. HOFFMAN AND D. E. NICHOLS. *[^{125}I]-2-(2,5-dimethoxy-4-iodophenyl)aminoethane ([^{125}I]-2C-I) as a label for the 5-HT$_2$ receptor in rat frontal cortex.* PHARMACOL BIOCHEM BEHAV **35**(1) 211–217, 1990.—Recent studies of 5-HT$_2$ receptor binding have involved the use of radiolabeled agonists. This report describes the use of [^{125}I]-2-(2,5-dimethoxy-4-iodophenyl)aminoethane ([^{125}I]-2C-I) as a label for low-density 5-HT$_2$ agonist binding sites. A nonhydrolyzable analog of GTP, GppNHp, was found to inhibit the high affinity binding of [^{125}I]-2C-I. 5-HT and several 5-HT$_2$ agonists and antagonists displayed high affinity for this site. In addition, a significant decrease in the B_{max} value, but not the K_D for [^{125}I]-2C-I was observed at 37°C as compared to that observed at 24°C. Several structure-activity relationships were investigated for displacement of [^{125}I]-2C-I, and the results are consistent with the importance of this receptor in the mechanism of action of hallucinogens. This study demonstrates the utility of [^{125}I]-2C-I as a novel radioligand and provides further data that the 5-HT$_2$ receptor is significantly linked to hallucinogenic activity for several compounds.

[^{125}I]-2-(2,5-dimethoxy-4-iodophenyl)aminoethane ([^{125}I]-2C-I) Receptor binding 5-HT$_2$ receptor Hallucinogens

UNTIL recently, the 5-HT$_2$ receptor has typically been labeled with 5-HT$_2$ antagonists such as [^{3}H]-ketanserin (15) or a nonselective partial agonist such as [^{3}H]-LSD (30). Early reports suggested that many 5-HT agonists, including serotonin itself, actually had a low affinity for the 5-HT$_2$ site (15). However, experiments by Battaglia and co-workers (1) revealed that some 5-HT$_2$ agonists displace [^{3}H]-ketanserin with both a low and high affinity component (17,35). Addition of GTP in those experiments eliminated the high affinity component, as might be expected for a 5-HT$_2$ receptor linked to GTP-dependent inositol triphosphate breakdown (32). These results suggested that the 5-HT$_2$ receptor might have a subtype or state of the receptor with high affinity for 5-HT$_2$ agonists.

Glennon and co-workers (10) found that 5-HT$_2$ antagonists block the discriminative stimulus effects of certain phenylisopropylamine hallucinogens. Subsequently, 5-HT$_2$ affinity, measured using [^{3}H]-ketanserin as the receptor label, was found to correlate with stimulus potency in a series of 4-substituted-2,5-dimethoxyphenylisopropylamines, in rats trained to discriminate the hallucinogen 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) from saline (8). This led to an investigation of the binding characteristics of [^{3}H]-1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane ([^{3}H]-DOB), another potent hallucinogenic phenylisopropylamine (36). [^{3}H]-DOB binding was found to be sensitive to nonhydrolyzable analogs of GTP, with a relatively low B_{max} (16). Recently, Titeler and co-workers (38) also reported a correlation between 5-HT$_2$ affinity, measured using [^{3}H]-DOB, and drug discrimination potency in DOM-trained rats.

Due to the low density of the high affinity sites and the low specific activity of the tritiated ligand, a large amount of tissue was required in binding experiments with [^{3}H]-DOB, and long exposure times would be necessary for autoradiography studies. This led us to examine the binding properties of the optical isomers of an [^{125}I]-labeled DOB analog 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ([^{125}I]-DOI) (14). McKenna and co-workers (18,19), and McKenna and Saavedra (20) also used this material for autoradiography experiments. In these studies the high specific activity [^{125}I] radiolabel was found to be an aid in labeling low density 5-HT$_2$ agonist sites. However, differences in affinity and receptor density were found between the *R*- and *S*-isomers of

[1]Present address: Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.
[2]Requests for reprints should be addressed to Dr. D. E. Nichols, Department of Medicinal Chemistry, Purdue University, West Lafayette, IN 47907.

ABBREVIATIONS

2C-I	2-(2,5-dimethoxy-4-iodophenyl)aminoethane
DOB	1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane
DOI	1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane
5-HT	5-hydroxytryptamine (serotonin)
5-MT	5-methoxytryptamine
5-MeO-AMT	5-methoxy-α-methyltryptamine
DMCPA	*trans*-2-(2,5-dimethoxy-4-methylphenyl) cyclopropylamine
8-OH-DPAT	8-hydroxy-2-dipropylaminotetralin
TFMPP	meta-trifluoromethylphenylpiperazine
GppNHp	guanosine 5'-(β,γ-imido)triphosphate

[^{125}I]-DOI, including a nearly two-fold decrease in affinity and B_{max} for the less active *S*-isomer in rat frontal cortex (14). Autoradiography experiments also indicated a different density of receptor sites labeled with *R*- and *S*-[^{125}I]-DOI in rat brain (18). Therefore, the optically pure more active *R*-isomer of [^{125}I]-DOI should be used as a radioligand. Alternatively, the use of an achiral analog might be advantageous as a new 5-HT_2 agonist receptor ligand.

In our continuing efforts to characterize the structure-activity relationships of hallucinogens, it was found that the α-desmethyl analog of DOI [2-(2,5-dimethoxy-4-iodophenyl)aminoethane; 2C-I] had an affinity very similar to the *R*-, and significantly greater than the *S*-isomer of DOI, which is in agreement with drug discrimination results. In DOM-trained rats, 2C-I is approximately equipotent to *R*-DOI (6,9). In experiments with rats trained to discriminate LSD from saline, it has been shown that 2C-I substitutes for LSD, and is approximately 100-fold less potent than LSD (28). Oberlender (28) also found that a 30-min pretreatment with 5 mg/kg of ketanserin completely blocked the drug-appropriate responding to a dose of 2C-I that gave 100% drug responding in LSD-trained rats. Therefore, the discriminative stimulus of 2C-I is similar to DOM and related phenylisopropylamine hallucinogens, and appears to be mediated through the 5-HT_2 receptor (10). This led to the synthesis of [^{125}I]-2C-I and its initial characterization, which is also presented here. This report provides evidence that [^{125}I]-2C-I binds specifically to the same GTP-sensitive state of the 5-HT_2 receptor as does *R*-[^{125}I]-DOI and *R,S*-[^{3}H]-DOB.

METHOD

Materials

R-[^{125}I]-DOI was synthesized by the procedure of Mathis *et al.* (23) at a specific activity of 1000 Ci/mmol. Ketanserin, 5-HT creatinine sulfate, 5-methoxytryptamine·HCl, pargyline·HCl, pindolol, GTP, GppNHp and GMP were purchased from Sigma Chemical Co. (St. Louis, MO), and TFMPP·HCl and spiperone were obtained from Research Biochemicals Inc. (Natick, MA). Ketanserin tartrate was graciously provided by Janssen Pharmaceutica (Beerse, Belgium), BOL, LSD, DMT and psilocin were acquired from the National Institute on Drug Abuse, 8-OH-DPAT from the Eli Lilly Laboratories (Indianapolis, IN), BL-3912A from Bristol Laboratories (Syracuse, NY). *R*- and *S*-DOI·HCl, *R*- and *S*-DOB·HCl, *R*-DOM·HCl, N(6)-ethyl-norLSD, N(6)-allyl-norLSD and other displacing ligands were synthesized in our laboratories.

Radiochemical Synthesis of [^{125}I]-2C-I

The radiosynthesis of 2-(2,5-dimethoxy-4-iodophenyl)aminoethane ([^{125}I]-2C-I) was conducted in a manner similar to that of [^{125}I]-labeled DOI (23). To 1 mg of 2,5-dimethoxyphenethylamine (4) contained in a Reacti-Vial (Pierce) equipped with a magnetic stirrer was added 300 μl of 2 M phosphoric acid, 25 mCi of sodium [^{125}I]-iodide (New England Nuclear) in ca. 50 μl of 0.1 M NaOH and 0.4 mg chloramine-T (Aldrich) in 20 μl H_2O. The vial was capped and the solution stirred at room temperature for 30 min. The reaction was quenched with 10 mg of sodium metabisulfite, and the solution made neutral with NaOH. The solution was filtered (Millipore HV, 0.45 μm) and the filter was washed with 1 ml of methanol. The filtrate was injected onto a Waters C18 analytical column and eluted with methanol/water (35/65) buffered to pH 7.6 with 0.1% triethylamine/phosphoric acid. The eluent fraction containing [^{125}I]-2C-I was collected and concentrated under an N_2 stream with gentle heating and reinjected onto the HPLC for a second purification. The overall radiochemical yield of [^{125}I]-2C-I was 80%, and the radiochemical purity of the product was greater than 99.9%. The specific activity of the product was 1400 Ci/mmole, as determined by UV quantitation of the mass peak co-eluting with [^{125}I]-2C-I on the second HPLC purification procedure.

Tissue Preparation

The tissue for the receptor binding studies was prepared according to the procedure of Battaglia and co-workers (1) with minor modifications. The frontal cortex region from 20 to 30 male Sprague-Dawley rats (175–199 g) was rapidly removed over ice. Tissue was homogenized in 4 volumes of 0.32 M sucrose in a Brinkman Polytron (setting 6 for 20 sec × 2). The homogenate was centrifuged (36,500 × g for 10 min), and the pellet was rehomogenized in the same volume of 0.32 M sucrose. Aliquots of 4.5 ml were stored at −70°C for up to three weeks.

On the day of the experiment, a tissue aliquot was thawed slowly in ice water and diluted 1 to 20 with 50 mM Tris HCl (pH = 7.4). The homogenate was incubated at 37°C for 10 min and centrifuged at 36,500 × g for 10 min. After a second centrifugation the final pellet was resuspended in 50 mM Tris HCl with 0.5 mM Na_2EDTA, 10 mM $MgCl_2$, 0.1% Na ascorbate, and 10 μM pargyline HCl (pH = 7.4). A second preincubation for 10 min at 37°C was conducted and the tissue was then allowed to cool in an ice bath.

Receptor Binding Procedures

All experiments were performed with triplicate determinations using the buffer described above, to which 200–400 μg protein was added, in a final volume of 1 ml. The amount of protein was determined according to the procedure of Bradford (3). Buffer, competing ligand and radioligand were added first to the test tubes. In saturation and displacement experiments, incubations were allowed to reach equilibrium in a shaking bath at 24°C or 37°C for 30 min as indicated, and were then filtered with a Brandel cell harvester modified for receptor binding studies. Filtration through Whatman GF/C filters was followed by two 3-second washes with ice-cold Tris buffer. The filters were counted on a Beckman 5500 gamma counter at an efficiency of 79%.

Saturation experiments were carried out by varying the concentration of unlabeled ligand with a constant amount of radioligand, according to the procedure described by Hamon and co-workers (11) and McPherson (21) and reported in our previous studies with *R*-[^{125}I]-DOI (14). In effect, increasing concentrations of radioligand, at decreasing specific activity were used, based on the picomoles of cold ligand added and the DPM's of radioligand added to each tube. This method is accurate when the

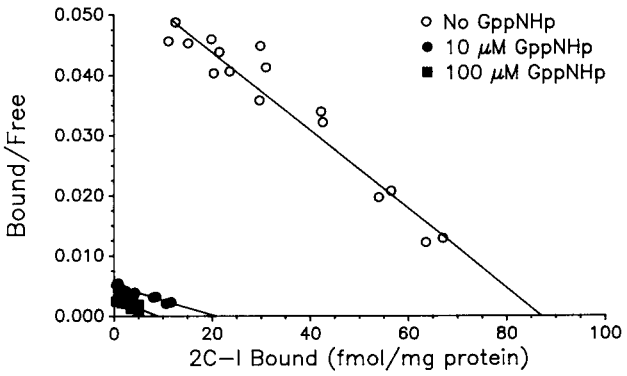

FIG. 1. The effect of GppNHp on the Scatchard plot of [^{125}I]-2C-I binding. As measured by total DPM's added, 0.25 nM [^{125}I]-2C-I was diluted with 0.1 to 5 nM 2C-I with and without 10 or 100 μM GppNHp added just prior to the 30-min incubation at 24°C. Each line represents the least squares analysis of a single site model. There was a decrease in the percent specific binding from 71.4% with control (○) to 32.8% and 22.2% when 10 μM GppNHp (●) or 100 μM GppNHp (■) was added, respectively. The representative values as mean ± S.E.M. (n) are: with no GppNHp added, $K_D = 1.52 \pm 0.10$ nM and $B_{max} = 87.0 \pm 3.8$ fmol/mg protein (3); with 10 μM GppNHp added, $K_D = 4.24 \pm 0.40$ nM and $B_{max} = 20.7 \pm 1.6$ fmol/mg protein (3); with 100 μM GppNHp added, $K_D = 3.17 \pm 0.83$ nM and $B_{max} = 8.9 \pm 1.8$ fmol/mg protein (2). The B_{max} values were significantly decreased with the addition of either 10 or 100 μM GppNHp ($p<0.001$, Students' t-test vs. control experiments). All determinations were run in triplicate.

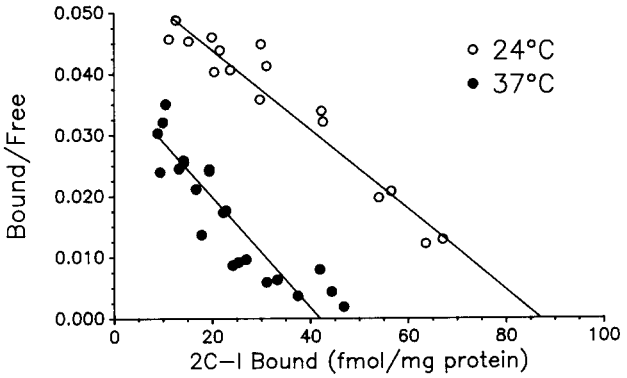

FIG. 2. The effect of equilibrium temperature on [^{125}I]-2C-I binding. As measured by total DPM's added, 0.25 nM [^{125}I]-2C-I was diluted with 0.1 to 5 nM 2C-I and incubated for 30 min at 24°C (○) or 37°C (●). Each line represents the Scatchard analysis for a least squares fit to a single site model as indicated by the Hill coefficients and computer assisted least squares analysis. There was no significant change in the percent specific binding or Hill coefficient for the Scatchard plots at 24°C and 37°C. There was a slight, but nonsignificant decrease in the K_D value at 37°C as compared to 24°C (1.11 ± 0.13 nM vs. 1.52 ± 0.10 nM). The B_{max} value was significantly decreased at 37°C as compared to 24°C ($p<0.001$, Students' t-test). The B_{max} values at 24°C and 37°C were 87.0 ± 3.8 fmol/mg protein and 41.9 ± 2.9 fmol/mg protein, respectively. All determinations were run in triplicate.

unlabeled and labeled ligands have identical physiochemical properties (27) as in this case. Approximately 0.25 nM [^{125}I]-2C-I was used in all experiments, as measured by the DPMs added. Unlabeled 2C-I (0.1 to 5 nM) was added to give varying concentrations for saturation experiments. Specific binding was defined as that displaceable by 1 μM cinanserin. Nine to eleven concentrations in the linear portion of the sigmoidal curve were used in displacement experiments. In the guanine nucleotide experiments GppNHp was added just prior to the addition of tissue.

Data Analysis

Scatchard and competition binding data for each experiment were analyzed by a nonlinear least squares curve fitting procedure, as embodied in the EBDA and LIGAND software, adapted for the IBM PC by McPherson (22). The K_D, B_{max} and K_I values reported represent the average of 3 to 4 separate experiments, with triplicate determinations at varying concentrations for each experiment.

RESULTS

[^{125}I]-2C-I Scatchard Analysis

As seen in Fig. 1, the Scatchard plot for [^{125}I]-2C-I binding was linear over the concentration range studied. The Hill plot also indicated a single site model with a Hill coefficient of 0.98 ± 0.01. Computer assisted analysis confirmed a single site model of a K_D of 1.52 ± 0.10 nM and a B_{max} of 87.0 ± 3.8 fmol/mg protein. Approximately 71% of the total binding was specific at 0.2 to 0.25 nM [^{125}I]-2C-I. It should be noted that under the experimental conditions concentrations of 2C-I greater than 5 nM yielded counts below nonspecific binding. This would imply, as reported for *R*- and *S*-[^{125}I]-DOI (14) and as suggested for racemic [^{3}H]-DOI (7) that [^{125}I]-2C-I may bind to a low affinity site, in addition to the high affinity site reported above. The B_{max} value is in reasonable agreement with previous reports of the density of 5-HT$_2$ agonist receptors in rat frontal cortex [see, for example, (7, 14, 37)]. Also, the K_D of [^{125}I]-2C-I corresponds well to the K_I measured using *R*-[^{125}I]-DOI as the label and to the K_I of the *R*-, but not the *S*-isomer of DOI and DOB.

The addition of 10 or 100 μM GppNHp prior to incubation substantially affected the binding characteristics of [^{125}I]-2C-I. Preliminary experiments indicated these concentrations of GppNHp were required to reach a plateau of inhibition. Although this is a somewhat higher concentration than was employed by Lyon *et al.* (16) with [^{3}H]-DOB, differences in incubation conditions and tissue preparation may account for this discrepancy. A direct comparison of the tissue preparation used by Lyon and co-workers (16) and the present one indicated a slight, but statistically significant decrease in percent specific binding and B_{max} with the method of Lyon *et al.* (16), but no change was measured in the K_D (data not shown). Combined, these data led us to speculate that less GTP may be removed from the tissue when a single 15-min incubation with EDTA and $MgCl_2$ is utilized. However, these slight decreases with less vigorous incubation conditions are probably of no technical importance.

Addition of GppNHp substantially decreased the percent specific binding of 2C-I from 71.4% to 32.8% and 22.2% for 10 and 100 μM GppNHp, respectively. As seen in Fig. 1, the addition of 10 and 100 μM GppNHp significantly decreased the B_{max} value for 2C-I to 20.7 fmol/mg protein and 8.9 fmol/mg protein, respectively ($p<0.001$, Student's t-test vs. experiments with no GppNHp added). These results are very similar to those seen with radioligand agonist binding to other G-protein linked receptors, for example the α_2-adrenergic receptor (34). There was also a slight decrease in affinity, although this may be partially due to the large

TABLE 1

DISPLACEMENT DATA FOR [^{125}I]-2C-I

	R-[^{125}I]-DOI	[^{125}I]-2C-I		
	K_I[a] (nM)	K_I (nM)	Pseudo Hill Coefficient	(n)
5-HT	3.63 ± 0.14	3.98 ± 0.65	1.03 ± 0.06	(3)
5-MT	3.24 ± 0.72	6.66 ± 1.16	0.79 ± 0.08	(4)
R-DOI	1.54 ± 0.38	2.62 ± 0.11	0.93 ± 0.02	(3)
S-DOI	6.35 ± 1.40	4.37 ± 0.63	1.09 ± 0.08	(3)
2C-I	1.52 ± 0.36	1.52 ± 0.10	0.98 ± 0.01	(4)†
2C-B	—	0.78 ± 0.08	1.02 ± 0.14	(3)
R-5-MeO-AMT	46.7 ± 8.90	69.8 ± 10.4	0.82 ± 0.04	(3)
S-5-MeO-AMT	1.90 ± 0.75	4.18 ± 0.42	0.78 ± 0.08	(3)
1*R*,2*S*-DMCPA‡	—	2.16 ± 0.26	0.88 ± 0.09	(3)
1*S*,2*R*-DMCPA	—	21.6 ± 1.07	0.91 ± 0.12	(3)
DMT	—	56.6 ± 5.7	1.00 ± 0.03	(3)
5-MeO-DMT	—	15.4 ± 0.5	1.17 ± 0.11	(3)
Psilocin	—	15.6 ± 1.8	0.97 ± 0.05	(4)
Ketanserin	3.55 ± 0.67	3.55 ± 0.66	0.81 ± 0.10	(3)§
Cinanserin	6.96 ± 0.58	2.90 ± 0.51	0.94 ± 0.10	(3)
Spiperone	3.22 ± 0.13	3.39 ± 0.36	0.89 ± 0.02	(3)
Pindolol	—	16,741 ± 1101	1.35 ± 0.18	(3)
TFMPP	21.1 ± 2.59	33.9 ± 3.02	0.92 ± 0.03	(4)
RU-24969	—	68.7 ± 12.6	1.00 ± 0.12	(3)
8-OH-DPAT	8528 ± 586	1548 ± 430	1.03 ± 0.12	(4)
DA, NE and Histamine	>10,000	>10,000	—	

*K_I values are for displacement of *R*-[^{125}I]-DOI from the high affinity component of a two-site model, following the procedures of Johnson *et al.* (14) with minor modifications. Briefly, the incubations were allowed to equilibrate for 60 min at 24°C and specific binding was defined as that displaceable by 10 μM *R*-DOI. Under these conditions all ligands above including *R*-[^{125}I]-DOI showed a two-site displacement as indicated by pseudo Hill coefficient and computer least squares analysis.

†K_I value and Hill coefficient taken from saturation experiments and calculated by Scatchard analysis; these are included for comparative purposes.

‡DMCPA is *trans*-2-(2,5-dimethoxy-4-methylphenyl)cyclopropylamine.

§K_I values reported for the 5-HT_2 antagonists ketanserin, cinanserin and spiperone are for displacement at 37°C.

As measured by total DPM's added, 0.25 nM of [^{125}I]-2C-I was used in each experiment. Test tubes were allowed to come to equilibrium for 30 min at 24°C. The K_I values for displacement from *R*-[^{125}I]-DOI-labeled sites are included for comparison. Unless otherwise specified, pseudo Hill coefficient and computer least squares analysis indicated a single site displacement of [^{125}I]-2C-I. Values represent mean ± S.E.M. for the indicated number (n) of independent experiments, with 9 to 11 concentrations of displacing ligand and triplicate determinations at each concentration.

standard error associated with the lower percentage of specific binding seen in the presence of GppNHp. Earlier experiments with GTP yielded similar effects, but higher concentrations (1 mM) of GTP were required to give maximum inhibition of binding. As indicated from experiments by Hamon *et al.* (11) and suggested by Lyon *et al.* (16), this is most likely due to degradation of GTP during the incubation.

The percent of specific binding, Hill coefficient or the K_D for [^{125}I]-2C-I obtained from Scatchard analysis (Fig. 2) were not significantly different at the two different equilibrium temperatures examined. However, increasing the temperature did significantly decrease the B_{max} value from 87.0 ± 3.8 to 41.9 ± 2.9 fmol/mg protein ($p<0.001$, Student's *t*-test). The B_{max} at 37°C is closer to that reported for other 5-HT_2 agonist radioligands under similar conditions [for example, (7)].

Displacement of [^{125}I]-2C-I by Various Ligands

As seen in Table 1, the K_I values for a number of displacing ligands closely parallel those measured using [^{3}H]-DOB (16). In addition, the K_I values with [^{125}I]-2C-I correspond well to those found in our laboratory using procedures previously reported with *R*-[^{125}I]-DOI as a radioligand (14). The K_I values reported for displacement from *R*-[^{125}I]-DOI labeled sites are included for comparison. Stereoselectivity was demonstrated for the *R*- and *S*-isomers of DOI, 5-methoxy-α-methyltryptamine (5-MeO-AMT) and the isomers of *trans*-2-(2,5-dimethoxy-4-methylphenyl)cyclopropylamine, DMCPA (26). Also *R*-DOB was found to have a higher affinity than its *S*-enantiomer for *R*-[^{125}I]-DOI-labeled receptor sites ($K_I = 1.40 \pm 0.20$ and 7.59 ± 1.73 nM, respectively; unpublished results). It is interesting to note that a 10- to 15-fold

selectivity was demonstrated for the more active *S*-5-MeO-AMT and 1*R*,2*S*-DMCPA while only an approximately 2-fold selectivity was seen for the *R*-enantiomer of DOI. In addition, the nonhallucinogenic alpha-ethyl analog of *R*-DOM, BL3912A, has a substantial decrease in affinity for *R*-[^{125}I]-DOI labeled sites (K_I = 340 ± 47 and 5.06 ± 1.99 nM for *R*-BL3912A and *R*-DOM, respectively; unpublished results).

Unless otherwise indicated, pseudo Hill coefficients were not significantly different from unity, and computer-assisted least squares analysis confirmed a single site model for displacement. Displacement of [^{125}I]-2C-I by the 5-HT$_2$ antagonists ketanserin, cinanserin and spiperone at 37°C yielded a single high affinity site (see Table 1). However, at 24°C, these 5-HT$_2$ antagonists yielded a two-site displacement (data not shown). The 5-HT$_2$ antagonist K_I values at 37°C correspond well to those previously published [see, for example, (16,37)].

DISCUSSION

From the Scatchard analysis (Figs. 1 and 2) it is clear that [^{125}I]-2C-I binds with high affinity to a single site in rat frontal cortex. The displacement profile presented in Table 1 further suggests that this receptor site is the same as that labeled by *R*-[^{125}I]-DOI. In fact, a linear regression analysis of K_I values using [^{125}I]-2C-I displacement on the K_I using *R*-[^{125}I]-DOI displacement gave an R>.99 (n=9). Similarly, a linear regression of the present data on the[^{3}H]-DOB displacement data of Lyon and co-workers (16) also yielded an R>.99 (n=9). [^{125}I]-2C-I binding was sensitive to guanine nucleotides (Fig. 1) as was that of [^{3}H]-DOB (16).

However, there is some indication from the lower B_{max} values obtained using [^{3}H]-DOB (16) and *R*-[^{77}Br]-DOB (40) that DOB may only bind to a subpopulation of the sites labeled by [^{125}I]-2C-I. This discrepancy is partially explained by the effect of equilibrium temperature on the B_{max} value for [^{125}I]-2C-I. As seen in Fig. 2, the B_{max} is halved at 37°C as compared to that seen at 24°C, with no significant change in the K_D or percent specific binding. In addition, the structural similarity and the similar pharmacological profile of DOI and DOB in rats (9), and the apparent significance of the 5-HT$_2$ receptor in their mechanism of action (8), would suggest that these ligands should bind to a common population of 5-HT$_2$ receptors.

Some controversy presently exists in the literature as to the identity of the receptor labeled by these phenethylamine hallucinogens. For example, the higher affinity of 5-HT$_2$ agonists for the [^{125}I]-2C-I-labeled sites, seen also with [^{3}H]-DOB (16,37) and *R*-[^{125}I]-DOI (14) might suggest that, as stated by Wang *et al.* (40), "classification of these sites as a subtype of 5-HT$_2$ binding sites may be premature." However, the two-component displacement by 5-HT$_2$ agonists at [^{3}H]-ketanserin sites (1, 17, 35), and the displacement profiles for these radiolabeled phenethylamine hallucinogens, strongly implicate the 5-HT$_2$ receptor. The present data further support this by demonstrating the low affinity of non-5-HT$_2$ ligands for the [^{125}I]-2C-I labeled site. For example, dopamine, norepinephrine and histamine all showed low affinity. The 5-HT$_{1A}$/5-HT$_{1B}$ antagonist, pindolol, as well as the 5-HT$_{1A}$ agonist 8-OH-DPAT (Table 1) and the 5-HT$_{1B}$ agonist RU-24969 also possess low affinity for [^{125}I]-2C-I labeled sites. Therefore, evidence from earlier work, and the present data, would suggest that radiolabeled DOI, 2C-I and DOB all bind to the same site, which may be some subpopulation of the 5-HT$_2$ receptor.

From Fig. 2, it would appear that the number of [^{125}I]-2C-I binding sites is a temperature-dependent phenomenon. Similar temperature dependency has been seen with other G-protein linked receptors. For example, Covents and co-workers (5) found that the dissociation rate constant was significantly greater for (−)-enpinephrine binding to the α_2-adrenergic receptor at 37°C, as compared to that at 24°C. Indeed, the decreased B_{max} for [^{125}I]-2C-I at 37°C as compared to that at 24°C, may be explained by analogies to the G-protein linked α_2-adrenergic receptor model proposed by Thomsen and co-workers (34). However, the validity of that model cannot be assessed for the 5-HT$_2$ receptor, based solely upon non-functional receptor assays such as those reported here.

Whether the receptor labeled by these phenethylamines is the "agonist state" of the 5-HT$_2$ receptor as originally proposed for [^{3}H]-DOB (16) or a separate subtype of the 5-HT$_2$ receptor as recently suggested for [^{77}Br]-DOB (29), the involvement of this receptor in the hallucinogenic effects of these drugs is strongly implicated. As previously mentioned, there are high correlations between the affinity for this 5-HT$_2$ receptor and the potency of hallucinogens in the drug discrimination paradigm [for example, see, (38)]. This is further supported in the present study by the enantioselectivity demonstrated by several different structural classes of hallocinogens. For example, the more potent *R*-enantiomer of DOI showed a two-fold higher affinity for [^{125}I]-2C-I labeled sites than its *S*-enantiomer. Similarly, *R*-DOB had a higher affinity for *R*-[^{125}I]-DOI-labeled sites than *S*-DOB. Further, the *S*-enantiomer of the tryptamine hallucinogen, 5-MeO-AMT demonstrated stereoselectivity (*S*>*R*) in agreement with its observed activity in humans (A. T. Shulgin, unpublished results). As seen in Table 1, the more active 1*R*,2*S*-enantiomer of *trans*-2-(2,5-dimethoxy-4-methylphenyl)cyclopropylamine, DMCPA (26), also showed stereoselectivity for the [^{125}I]-2C-I labeled site.

The importance of the receptor labeled with these phenethylamine hallucinogens is further evident by examining several structure activity relationships. For example, the nonhallucinogenic α-ethyl homologue of *R*-DOM, BL-3912A (39), has a K_I value of 340 ± 47 nM for *R*-[^{125}I]-DOI-labeled sites, which is over fifty-fold higher than the K_I value found for *R*-DOM under similar conditions (unpublished results). The N(6)-ethyl (ETHLAD) and N(6)-allyl (ALLAD) analogs of LSD have been reported to be more potent than LSD in the rat and in humans (12, 13, 24). ETHLAD, ALLAD and LSD were all found to have high affinity for the *R*-[^{125}I]-DOI-labeled site (5.11 ± 0.34, 3.36 ± 0.75 and 5.17 ± 0.96 nM, respectively; unpublished results). This does not imply, however, that LSD or its analogs have the same mechanism of action as the phenethylamine hallucinogens. For example, 2-bromo-LSD (BOL) also has a high affinity for this receptor site (4.56 ± 0.30 nM, unpublished results), but is known to be an antagonist of hallucinogenic activity. In addition, there is a growing body of evidence to suggest that LSD is actually a partial agonist/antagonist at 5-HT$_2$ receptors (2, 30, 31, 33). Therefore, while the receptor labeled with phenethylamine hallucinogens is implicated in hallucinogen action, as with most receptor binding assays the ability to displace [^{125}I]-2C-I or any other phenethylamine hallucinogen, is not predictive of hallucinogenic activity.

One advantage of these radioligands is the ability of radiolabeled agonists to identify structure activity relationships that are not apparent when a radiolabeled antagonist is utilized. For example, there was an apparent low affinity of the potent hallucinogen, 5-MeO-AMT for the 5-HT$_2$ receptor when [^{3}H]-ketanserin was used as a radioligand (25). In contrast, utilizing either *R*-[^{125}I]-DOI or [^{125}I]-2C-I as a receptor label revealed a less than 10 nM affinity for *S*-5-MeO-AMT and a 20-fold higher affinity than its less active *R*-enantiomer (see Table 1). Similarly, a comparison of 5-HT$_2$ receptor affinity for DMT, 5-MeO-DMT and psilocin suggests that either 4-hydroxylation or 5-methoxylation of the DMT nucleus results in a comparable increase in receptor affinity. Therefore, it appears that 4-hydroxylation in psilocin serves not merely to confer metabolic resistance and increase lipid solubility (24), but may also increase affinity for the agonist state

of the 5-HT_2 receptor. This enhancement of affinity is not observed with 4-hydroxylation when [^{3}H]-ketanserin is used as the 5-HT_2 ligand (25).

The obvious advantage of these new ligands is the use of the [^{125}I]-label, which provides much higher specific activity than [^{3}H]-ligands and a greater ability to measure low density 5-HT_2 agonist binding sites. In addition, and also of importance, is the fact that [^{125}I]-2C-I is an achiral compound, avoiding the problems associated with the synthesis of pure optical isomers and labeling a receptor with racemic ligands. Therefore, [^{125}I]-2C-I should prove to be a useful tool in binding affinity measurements for the 5-HT_2 receptor, as well as a useful ligand for in vitro autoradiography experiments. We further anticipate that the analogous bromo homologue, 2C-B, labeled with [^{3}H] or [^{77}Br], would be more advantageous as a radioligand than racemic [^{3}H]-DOB or [^{77}Br]-DOB.

ACKNOWLEDGEMENTS

We thank Lea Reisenman and Margaret Debska for excellent technical assistance. This work was supported by USPHS grant DA-02189, from the National Institute on Drug Abuse, and NIH grant NS 22899.

REFERENCES

1. Battaglia, G.; Shannon, M.; Titeler, M. Guanyl nucleotide and divalent cation regulation of cortical S_2 serotonin receptors. J. Neurochem. 43:1213–1219; 1984.
2. Bennett, J. P.; Snyder, S. H. Serotonin and lysergic acid diethylamide binding in rat brain membranes: Relationship to postsynaptic serotonin receptors. Mol. Pharmacol. 12:373–389; 1976.
3. Bradford, M. M. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254; 1976.
4. Cheng, A. C.; Castagnoli, N., Jr. Synthesis and physiochemical and neurotoxicity studies of 1-(4-substituted-2,5-dihydroxyphenyl)-2-aminoethane analogues of 6-hydroxydopamine. J. Med. Chem. 27:513–520; 1984.
5. Covents, A.; De Baker, J. J.-P.; Convents, D.; Vauquelin, G. Tight agonist binding may prevent the correct interpretation of agonist competition binding curves for α_2-adrenergic receptors. Mol. Pharmacol. 32:65–72; 1987.
6. Glennon, R. A. Discriminative stimulus properties of the serotonergic agent 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Life Sci. 39:825–830; 1986.
7. Glennon, R. A.; Seggel, M. R.; Soine, W. H.; Herrick-Davis, K.; Lyon, R. A.; Titeler, M. [^{125}I]-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane: An iodonated radioligand that specifically labels the agonist high-affinity state of 5-HT_2 serotonin receptors. J. Med. Chem. 31:5–7; 1988.
8. Glennon, R. A.; Titeler, M.; McKenney, J. D. Evidence for 5-HT_2 involvement in the mechanism of action of hallucinogenic agents. Life. Sci. 35:2505–2511; 1984.
9. Glennon, R. A.; Young, R.; Benington, F.; Morin, R. D. Behavioral and serotonin receptor properties of 4-substituted derivatives of the hallucinogen 1-(2,5-dimethoxyphenyl)-2-aminopropane. J. Med. Chem. 25:1163–1168; 1982.
10. Glennon, R. A.; Young, R.; Rosecrans, J. A. Antagonism of the effects of the hallucinogen DOM and the purported 5-HT agonist quipazine by 5-HT_2 antagonists. Eur. J. Pharmacol. 91:189–196; 1983.
11. Hamon, M.; Mallat, M.; Mestikawy, S. E.; Pasquier, A. Ca^{2+}-guanine nucleotide interactions in brain membranes. II. Characteristics of [^{3}H]-guanosine triphosphate and [^{3}H]-beta, gamma-imidoguanosine 5′-triphosphate binding and catabolism in the rat hippocampus and striatum. J. Neurochem. 38:162–172; 1982.
12. Hoffman, A. J. Ph.D. thesis, Purdue University; 1987.
13. Hoffman, A. J.; Nichols, D. E. Synthesis and LSD-like discriminative stimulus properties in a series of N(6)-alkyl norlysergic acid N,N-diethylamide derivatives. J. Med. Chem. 28:1252–1255; 1985.
14. Johnson, M. P.; Hoffman, A. J.; Nichols, D. E.; Mathis, C. A. Binding to the serotonin 5-HT_2 receptor by the enantiomers of [^{125}I]-DOI. Neuropharmacology 26:1803–1806; 1987.
15. Leysen, J. E.; Niemegeers, J. E.; Van Nueten, J. M.; Laduron, P. M. [^{3}H]-ketanserin (R 41 468), a selective [^{3}H]-ligand for serotonin$_2$ receptor binding sites. Mol. Pharmacol. 21:301–314; 1981.
16. Lyon, R. A.; Davis, K. H.; Titeler, M. [^{3}H]-DOB (4-bromo-2,5-dimethoxyphenylisopropylamine) labels a guanyl nucleotide-sensitive state of cortical 5-HT_2 receptors. Mol. Pharmacol. 31:194–199; 1987.
17. Lyon, R. A.; Titeler, M.; Seggel, M. R.; Glennon, R. A. Indolealkylamine analogs share 5-HT_2 binding characteristics with phenylalkylamine hallucinogens. Eur. J. Pharmacol. 145:291–297; 1988.
18. McKenna, D. J.; Mathis, C. A.; Shulgin, A. T.; Sargent, T., III; Saavedra, J. M. Autoradiographic localization of binding sites for [^{125}I]-DOI, a new psychotomimetic radioligand, in the rat brain. Eur. J. Pharmacol 137:289–290; 1987.
19. McKenna, D. J.; Nazarali, A. J.; Hoffman, A. J.; Nichols, D. E.; Mathis, C. A.; Saavedra, J. M. Common receptors for hallucinogens in rat brain: a comparative autoradiographic study using [^{125}I]-LSD and [^{125}I]-DOI, a new psychotomimetic radioligand. Brain Res. 476:45–56; 1989.
20. McKenna, D. J.; Saavedra, J. M. Autoradiography of LSD and 2,5-dimethoxyphenylisopropylamine psychotomimetics demonstrates regional, specific cross-displacement in the rat brain. Eur. J. Pharmacol. 142:313–315; 1987.
21. McPherson, G. A. A practical computer-based approach to the analysis of radioligand binding experiments. Comput. Prog. Biomed. 17:107–114; 1983.
22. McPherson, G. A. Analysis of radioligand binding experiments: a collection of computer programs for the IBM PC. J. Pharmacol. Methods 14:213–228; 1985.
23. Mathis, C. A.; Hoffman, A. J.; Nichols, D. E.; Shulgin, A. T. Synthesis of high-specific activity ^{125}I- and ^{123}I-labelled enantiomers of 2,5-dimethoxy-4-iodophenylisopropylamine (DOI). J. Label. Comp. Radiopharmaceut. 25:1255–1265; 1988.
24. Nichols, D. E. Studies of the relationship between molecular structure and hallucinogenic activity. Pharmacol. Biochem. Behav. 24:335–341; 1986.
25. Nichols, D. E.; Lloyd, D. H.; Johnson, M. P.; Hoffman, A. J. Synthesis and serotonin receptor affinities of a series of enantiomers of alpha-methyltryptamines: Evidence for the binding conformation of tryptamines at serotonin 5-HT_{1B} receptors. J. Med. Chem. 31: 1406–1412; 1988.
26. Nichols, D. E.; Woodard, R.; Hathaway, B. A.; Lowy, M. T.; Yim, G. K. W. Resolution and absolute configuration of *trans*-2-(2,5-dimethoxy-4-methylphenyl)cyclopropylamine, a potent hallucinogen analogue. J. Med. Chem. 22:458–460; 1979.
27. Nicosia, S. More about the misuse of Scatchard plots in binding studies. Pharmacol. Res. Commun. 20:733–737; 1988.
28. Oberlender, R. Ph.D. Thesis, Purdue University; 1989.
29. Peroutka, S. J.; Hamik, A.; Harrington, M. A.; Hoffman, A. J.; Mathis, C. A.; Pierce, P. A.; Wang, S. S.-H. (*R*)-(−)-[^{77}Br]4-bromo-2,5-dimethoxyamphetamine labels a novel 5-hydroxytryptamine binding site in brain membranes. Mol. Pharmacol. 34:537–542; 1988.
30. Peroutka, S. J.; Snyder, S. H. Multiple serotonin receptors: Differential binding of [^{3}H]-5-hydroxytryptamine, [^{3}H]-lysergic acid diethylamide and [^{3}H]-spiroperidol. Mol. Pharmacol. 16:687–699; 1979.
31. Sanders-Bush, E.; Burris, K. D.; Knoth, K. Lysergic acid diethylamide and 2,5-dimethoxy-4-methylamphetamine are partial agonists at serotonin receptors linked to phosphoinositide hydrolysis. J. Pharmacol. Exp. Ther. 246:924–928; 1988.
32. Sanders-Bush, E.; Conn, P. J. Effector systems coupled to serotonin receptors in brain: Serotonin stimulated phosphoinositide hydrolysis. Psychopharmacol. Bull. 22:829–836; 1986.
33. Sills, M. A.; Wolfe, B. B.; Frazer, A. Determination of selective and nonselective compounds for the 5-HT_{1A} and 5-HT_{1B} receptor subtypes in rat frontal cortex. J. Pharmacol. Exp. Ther. 231:480–487; 1984.

34. Thomsen, W. J.; Jacquez, J. A.; Neubig, R. R. Inhibition of adenylate cyclase is mediated by the high affinity conformation of the α_2-adrenergic receptor. Mol. Pharmacol. 34:814–822; 1988.
35. Titeler, M.; Battaglia, G.; Shannon, M. Guanine nucleotides modulate cortical S_2 serotonin receptors. J. Recept. Res. 4:705–712; 1984.
36. Titeler, M.;Herrick, K.; Lyon, R. A.; McKenney, J. D.; Glennon, R. A. [^{3}H]-DOB: a specific agonist radioligand for 5-HT$_2$ serotonin receptors. Eur. J. Pharmacol. 117:145–146; 1985.
37. Titeler, M.; Lyon, R. A.; Davis, K. H.; Glennon, R. A. Selectivity of serotonergic drugs for multiple brain serotonin receptors: Role of [^{3}H]-4-bromo-2,5-dimethoxyphenylisopropylamine ([^{3}H]-DOB), a 5-HT$_2$ agonist radioligand. Biochem. Pharmacol. 36:3265–3271; 1987.
38. Titeler, M.; Lyon, R. A.; Glennon, R. A. Radioligand binding evidence implicates the brain 5-HT$_2$ receptor as a site of action for LSD and phenylisopropylamine hallucinogens. Psychopharmacology (Berlin) 94:213–216; 1988.
39. Winter, J. C. Effects of the phenethylamine derivatives, BL-3912, fenfluramine and Sch-12679 in rats trained with LSD as a discriminative stimulus. Psychopharmacology (Berlin) 68:159–162; 1980.
40. Wang, S. S.-H.; Mathis, C. A.; Peroutka, S. J. *R*-(−)-2,5-dimethoxy-4-^{77}bromoamphetamine [^{77}Br-*R*-(−)DOB]: a novel radioligand which labels a 5-HT binding site subtype. Psychopharmacology (Berlin) 94:431–432; 1988.