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^{3}H-DOB (4-Bromo-2,5-Dimethoxyphenylisopropylamine) Labels a Guanyl Nucleotide-Sensitive State of Cortical 5-HT$_2$ Receptors

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SUMMARY

^{3}H-(±)-4-Bromo-2,5-dimethoxyphenylisopropylamine (^{3}H-DOB), a putative agonist radioligand, was synthesized and used to label 5-HT$_2$ receptors in a particulate fraction prepared from rat frontal cortex tissue homogenates. The specific binding (defined by the difference in ^{3}H-DOB binding in the presence and absence of 10^{-6} M cinanserin, a potent and specific 5-HT$_2$ antagonist) displayed high affinity ($K_D = 4.1 \times 10^{-10}$ M) and saturability with a B_{max} of 17.9 fmol/mg of protein. The distribution of specific ^{3}H-DOB binding in nine brain regions correlated closely with the distribution of ^{3}H-ketanserin (an antagonist radioligand)-labeled 5-HT$_2$ receptors. Competition studies in frontal cortex homogenates using a variety of compounds revealed a distinct 5-HT$_2$ receptor pharmacology. A series of 5-HT$_2$ antagonists exhibited high affinities in competition studies for specific ^{3}H-DOB binding. The absolute potencies of these antagonists as well as their order of potencies closely correlated with their potencies in competing for ^{3}H-ketanserin-labeled brain 5-HT$_2$ receptors. A series of 5-HT$_2$ agonists also exhibited high affinities in competition studies for specific ^{3}H-DOB binding. Although the order of potencies of these agonists was similar to their order in competing for ^{3}H-ketanserin-labeled brain 5-HT$_2$ receptors, the agonists displayed 10–100-fold higher affinities for the ^{3}H-DOB-labeled sites than for the ^{3}H-ketanserin-labeled sites. The level of specific ^{3}H-DOB binding in the frontal cortex homogenates was approximately 5% of the levels of ^{3}H-ketanserin-labeled 5-HT$_2$ receptors (358 fmol/mg of protein). Taken together, these results indicate that ^{3}H-DOB labels a subset of brain 5-HT$_2$ receptors that has high affinity for agonists as well as antagonists); ^{3}H-ketanserin appears to label both subsets of brain 5-HT$_2$ receptors. Antagonists apparently do not discriminate between these two subsets of 5-HT$_2$ receptors. ^{3}H-DOB specific binding to 5-HT$_2$ receptors was potently inhibited by guanosine 5′-(β,γ-imido)triphosphate and guanosine 5′-*O*-(3-thio)triphosphate (nonhydrolyzable derivatives of GTP) with IC$_{50}$ values of 42 and 21 nM, respectively, whereas adenosine 5′-(β,γ-imido)triphosphate and adenosine 5′-*O*-(3-thio)triphosphate (nonhydrolyzable derivatives of ATP) had no effect. In summary, ^{3}H-DOB specific binding displays the pharmacological characteristics of a 5-HT$_2$ receptor. Furthermore, the guanyl nucleotide sensitivity, the high affinity of agonists, and the relatively low number of sites labeled indicate that ^{3}H-DOB labels a high affinity state of the 5-HT$_2$ receptor that either preexists in the tissue homogenate or is induced due to the presence of the agonist. Presumably the agonist-induced high affinity state of the receptor involves a GTP-binding regulatory protein (N subunit).

In 1976 Bennett and Snyder (1) demonstrated high affinity binding of ^{3}H-serotonin (5-HT) to rat brain homogenates. In competition studies, this site demonstrated high affinities for tryptamine-like drugs and lysergic acid diethylamide, drugs believed to have serotonergic activity. In general, drugs believed to have serotonin receptor-blocking activity demonstrated relatively low affinity for the ^{3}H-serotonin-labeled site. In 1979 Leysen *et al.* (2) demonstrated high affinity binding of ^{3}H-spiperone, a dopamine and serotonin receptor antagonist, to rat cortical homogenates. Of the monoamine neurotransmitters, serotonin was the most potent inhibitor of the ^{3}H-spiperone binding. Serotonin receptor antagonists competed for this binding site with high affinity and with potencies that correlated with their potencies in blocking serotonergic behaviors in rats. In 1981 Peroutka *et al.* (3) named the high affinity ^{3}H-serotonin binding phenomenon "5-HT$_1$" and defined it as a serotonin receptor possessing high affinity for serotonin and other tryptamines, as well as for lysergic acid diethylamide, and low affinity for most classical antagonists. They named the ^{3}H-spiperone-binding site "5-HT$_2$" and defined it as a serotonin receptor possessing high affinity for most classical serotonin receptor antagonists and relatively low affinity for serotonin. Titeler and co-workers (4–6) demonstrated that the ^{3}H-ketanserin-labeled brain 5-HT$_2$ receptor appeared to exist in two states: one state appeared to have high affinity for agonists and the other appeared to have low affinity for agonists. Antagonists appeared unable to discriminate between the two states of the

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); DOB, 4-bromo-2,5-dimethoxyphenylisopropylamine; ^{3}H-DOB, ^{3}H-(±)-4-bromo-2,5-dimethoxyphenylisopropylamine; ^{3}H-8-OH-DPAT, ^{3}H-8-hydroxydipropylaminotetralin; EDTA, ethylenediaminetetraacetate; GppNHp, guanosine 5′-(β,γ-imido)triphosphate; GTPγS, guanosine 5′-*O*-(3-thio)triphosphate; AppNHp, adenosine 5′-(β,γ-imido)triphosphate; ATPγS, adenosine 5′-*O*-(3-thio)triphosphate.

receptor. Titler and co-workers (4–7) demonstrated that the interactions of agonists (and not antagonists) with the ^{3}H-ketanserin-labeled 5-HT$_2$ receptor was regulated by guanyl nucleotides, divalent cations, monovalent cations, and pH. It was concluded that the 5-HT$_2$ receptor appeared to be a two-state receptor presumably interacting with a GTP-binding regulatory protein (N subunit).

In 1983 it was demonstrated that a series of phenylisopropylamine hallucinogens competed for ^{3}H-ketanserin-labeled 5-HT$_2$ receptors with affinities that correlated closely with their potencies as hallucinogens in man and as discriminable stimuli in rats trained to recognize the hallucinogen 2,5-dimethoxyphenylisopropylamine (8, 9). 5-HT$_2$ antagonists blocked the phenylisopropylamine discrimination in rats; these results implied that the phenylisopropylamines were acting as agonists at the rat brain 5-HT$_2$ receptors (10). Among these phenylisopropylamines was the compound DOB. It was decided to radiolabel DOB in an attempt to produce a specific radiolabel probe of the 5-HT$_2$ receptor possessing classical agonist-like properties (for reviews see Refs. 11 and 12). Preliminary work from our laboratory has shown that ^{3}H-DOB appears to be a selective 5-HT$_2$ receptor agonist radioligand (13).

Materials and Methods

^{3}H-DOB (40 Ci/mmol) was custom synthesized by New England Nuclear; ^{3}H-ketanserin (76 Ci/mmol), ^{3}H-serotonin (23 Ci/mmol), and ^{3}H-8-OH-DPAT (120 Ci/mmol) were purchased from New England Nuclear. Guanyl and adenyl nucleotides were obtained from Sigma or Boehringer. Drugs used in competition studies were purchased from Sigma or Research Biochemicals Inc., kindly provided by Dr. Richard Glennon and Dr. Michael Kuhar, or obtained commercially. The tissue preparation was a modification of a procedure previously described (5). Nine regions of brain tissue from male Taconic Farms Sprague-Dawley rats (180–200 g) were dissected on ice according to the method of Glowinski and Iverson (14) and homogenized in ice-cold 50 mM Tris-HCl, 0.5 mM EDTA, 10 mM $MgCl_2$, pH 7.4 (1:10 w/v), and centrifuged at 30,000 × g for 15 min. The pellet was resuspended in buffer (1:30 w/v), incubated at 37° for 15 min, and then centrifuged at 30,000 × g for 10 min twice (with a resuspension between centrifugations). The final pellet was resuspended in 50 mM Tris-HCl, 0.5 mM EDTA, 10 mM $MgCl_2$, 0.1% ascorbate, and 10^{-5} M pargyline.

Assays were performed in triplicate three times in a 2.0-ml volume containing 20 mg wet weight of tissue (which was added last) for ^{3}H-DOB experiments and 3 mg of tissue for ^{3}H-ketanserin experiments. ^{3}H-DOB and ^{3}H-ketanserin saturation analyses were performed over 3 log units of ^{3}H-ligand concentration using 1 μM cinanserin to define nonspecific binding. In parallel saturation experiments, actual free ^{3}H-DOB levels were directly determined by centrifugation of the incubation medium and assaying the supernatants. The difference in actual free ^{3}H-DOB versus the total added ^{3}H-DOB was less than 10% and no differences were found in Scatchard analysis of the data. Competition experiments were performed using 4 × 10^{-10} M ^{3}H-DOB; at this concentration 54% of the total binding was specific. For the competition experiments a mean of 1183 ± 37 dpm was detected in the tubes containing only ^{3}H-DOB and membranes and a mean of 549 ± 16 dpm in tubes containing ^{3}H-DOB, membranes, and excess cinanserin. There was no significant difference in total or specific binding of 0.4 nM ^{3}H-DOB at 24° as opposed to 37°. ^{3}H-Ketanserin competition experiments were performed using 4 × 10^{-10} M ^{3}H-ligand and, in each case, 11 concentrations of competing drug were used. To obtain kinetic constants, samples of ^{3}H-DOB total and nonspecific binding were assayed at various time points during association and dissociation; dissociation was initiated by adding 1 μM cinanserin. 5-HT$_1$A competition experiments were performed as above using 1 × 10^{-10} M ^{3}H-8-OH-DPAT with 1 μM 8-OH-DPAT used to define nonspecific binding in 8 mg wet weight of hippocampal membranes. 5-HT$_1$B competition experiments were performed using 1.5 × 10^{-9} M ^{3}H-5-HT in the presence of 100 nM 8-OH-DPAT and 100 nM mesulergine, with 1 μM 5-HT used to define nonspecific binding in 10 mg wet weight of striatal membranes. Tubes were incubated for 15 min at 37°, filtered on Schleicher and Schuell (Keene, NH) glass fiber filters (presoaked in 0.1% polyethyleneimine), and washed with 10 ml of ice-cold buffer. The filters were counted by liquid scintillation spectrometry (Beckman 3801) in 5 ml of aqueous counting scintillant (Formula 963, New England Nuclear), following 6 hr of equilibration at an efficiency of 50%. Protein determinations were performed following the method of Lowry *et al.* (15) using bovine serum albumin as a standard.

Saturation and competition experiments were analyzed using an updated version of the program EBDA (16), to obtain equilibrium dissociation constants (K_D), B_{max} values, Hill coefficients, and IC_{50} values. K_i values for competition experiments were obtained using the Cheng-Prusoff equation (17). Kinetic constants were obtained using the program RS/1 (BBN Software).

Results

As shown in Fig. 1A, specific ^{3}H-DOB binding was saturable and demonstrated high affinity in homogenates of rat frontal

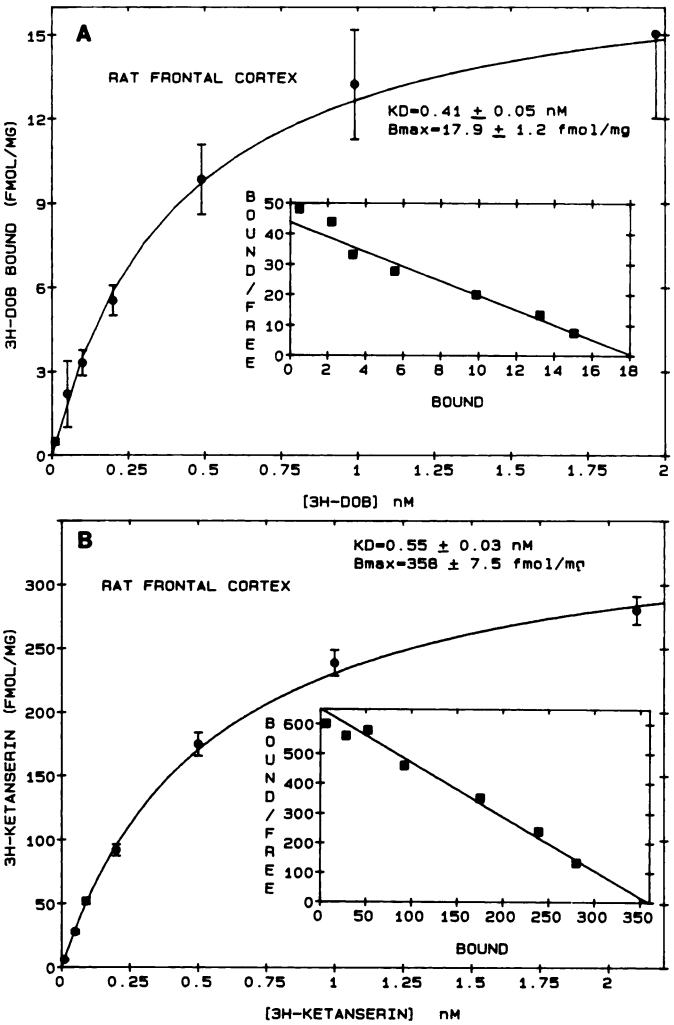

Fig. 1. Scatchard analyses of ^{3}H-DOB (A)- and ^{3}H-ketanserin (B)-labeled 5-HT$_2$ receptors in rat brain homogenates. Cinanserin (1 μM) was used to define nonspecific binding. The points plotted are the result of three experiments performed in triplicate. Standard errors are less than 5% for each point.

TABLE 1

Rat brain distribution of specific ^{3}H-ketanserin and ^{3}H-DOB binding

Cinanserin (1 μM) was used to define nonspecific binding. Values reported are the mean and standard error of three independent experiments. B_{max} values are reported in fmol/mg of protein [Lowry method (15)] and K_D values are reported in nM. In each case, the Hill coefficients did not differ significantly from unity. The correlation coefficient for ^{3}H-ketanserin versus ^{3}H-DOB B_{max} values equals 0.96.

Region	^{3}H-Ketanserin		^{3}H-DOB	
	B_{max}	K_D	B_{max}	K_D
1. Frontal cortex	361 ± 15	0.55 ± 0.04	17.9 ± 1.2	0.41 ± 0.05
2. Rest of cortex	195 ± 21	0.78 ± 0.05	8.9 ± 0.88	0.55 ± 0.10
3. Striatum	227 ± 23	0.68 ± 0.04	8.4 ± 0.94	0.34 ± 0.07
4. Hippocampus	58 ± 5	0.66 ± 0.04	5.7 ± 0.86	0.51 ± 0.09
5. Hypothalamus	69 ± 3	0.79 ± 0.09	5.0 ± 0.30	0.34 ± 0.06
6. Diencephalon	66 ± 13	0.82 ± 0.11	4.6 ± 0.58	0.51 ± 0.09
7. Midbrain	45 ± 7	0.99 ± 0.02	4.0 ± 0.18	0.60 ± 0.07
8. Brainstem	37 ± 4	0.97 ± 0.10	3.1 ± 0.44	0.61 ± 0.15
9. Cerebellum	8 ± 2	0.63 ± 0.09	2.7 ± 0.43	0.42 ± 0.10

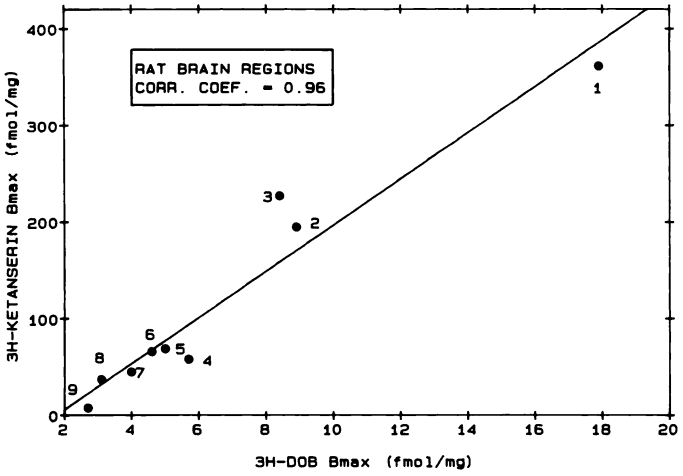

Fig. 2. Correlation of ^{3}H-ketanserin and ^{3}H-DOB specific binding in rat brain regions. Saturation experiments were performed as described in Materials and Methods. Standard errors are less than 5% for each point. Correlation coefficient equals 0.96.

cortex. Scatchard analysis of the saturation data indicated a B_{max} of 17.9 fmol/mg of protein and a K_D of 4.1×10^{-10} M (Fig. 1A, *inset*). In parallel experiments in rat frontal cortex, ^{3}H-ketanserin specific binding demonstrated a B_{max} of 358 fmol/mg of protein (Fig. 1B). Boiling of rat frontal cortical tissue for 5 min before assay resulted in the complete loss of specific binding (data not shown). Throughout the brain regions examined (Table 1, ^{3}H-DOB binding was consistently present in low levels relative to the levels of ^{3}H-ketanserin specific binding. The relative distribution of specific ^{3}H-DOB binding throughout the brain regions correlated closely with the levels of specific ^{3}H-ketanserin binding ($r = 0.96$, Fig. 2) and frontal cortical tissue contained the highest levels of both ^{3}H-DOB and ^{3}H-ketanserin specific binding (Table 1). It should be noted that the pharmacological characteristics of specific ^{3}H-DOB binding in brain regions other than the frontal cortical tissue were not examined; however, DOB has not been found to have significant affinity for any other monoamine receptor in preliminary screens.[1] Furthermore, racemic DOB has low affinity for 5-HT$_1$A and 5-HT$_1$B receptors (Table 2).

Fig. 3 demonstrates the association and dissociation kinetics of 2×10^{-10} M ^{3}H-DOB binding to 20 mg wet weight of rat

[1] R. Glennon, personal communication.

TABLE 2

(±)-DOB affinities for agonist-labeled 5-HT$_1$ receptors

Eleven concentrations of racemic DOB were competed for ^{3}H-agonist-labeled 5-HT$_1$ receptors (see Materials and Methods). Values reported are the mean and standard error of three independent experiments performed in triplicate. K_i values were obtained using the Cheng-Prusoff equation (17).

Receptor	K_i	Hill:
	nM	
5-HT$_1$A	3770 ± 118	0.94 ± 0.03
5-HT$_1$B	831 ± 37	0.92 ± 0.02

frontal cortical tissue. The association constant (k_a) calculated from a first order fit of the observed data was 1.5×10^9 M^{-1} min^{-1} and the dissociation constant (k_d) was 0.26 min^{-1}. The calculated equilibrium dissociation constant ($K_D = k_d/k_a$) was 1.73×10^{-10} M, which is very close to the K_D calculated from the equilibrium saturation experiments (Fig. 1A).

The pharmacological characteristics of specific ^{3}H-DOB binding to rat frontal cortex membranes are displayed in Fig. 4 and Table 2. Fig. 4 displays representative competition curves for agonists and antagonists; Table 2 lists the K_i values and Hill coefficients derived from competition experiments. Nonserotonergic compounds showed similar low affinities in competing for ^{3}H-ketanserin and ^{3}H-DOB binding. Of special interest was the far higher affinities of serotonin receptor agonists in ^{3}H-DOB competition experiments relative to their affinities displayed in ^{3}H-ketanserin competition experiments. (Table 3). However, agonist competition for ^{3}H-DOB-labeled receptors correlated strongly with computer-generated high affinity agonist competition for ^{3}H-ketanserin-labeled receptors ($r = 0.92$). In contrast, antagonists appeared to have similar affinities in competition experiments using ^{3}H-DOB or ^{3}H-ketanserin (Table 3), and, again, a strong correlation was found ($r = 0.96$, Fig. 5).

Fig. 6 displays the effect of the nonhydrolyzable analogs of GTP, GppNHp and GTPγS, on specific ^{3}H-DOB binding. The EC_{50} values in inhibiting specific ^{3}H-DOB binding were 42 and 21 nM, respectively, and the Hill coefficients of the concentration-inhibition curves were significantly less than unity (Table 4). GppNHp and GTPγS inhibited 70% of specific ^{3}H-DOB binding. The adenyl analogs, AppNHp and ATPγS, showed no appreciable effect on specific ^{3}H-DOB binding (Fig. 6).

Discussion

The pharmacological characteristics of specific ^{3}H-DOB binding indicate that this putative agonist radioligand is label-

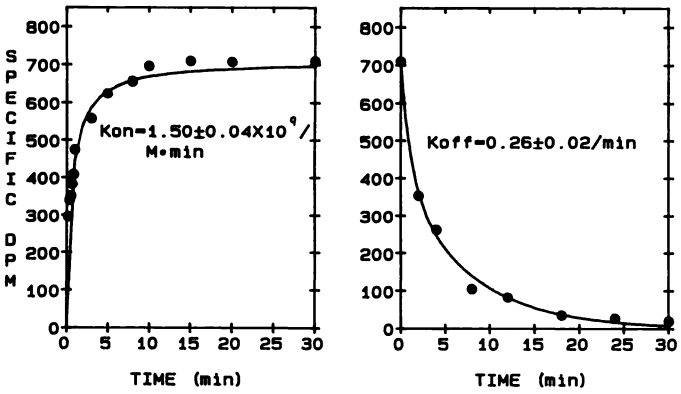

Fig. 3. ^{3}H-DOB association and dissociation kinetics. ^{3}H-DOB (0.2 nM) was incubated with rat frontal cortical membranes as described in Materials and Methods. Computer-derived first order rate constants are shown in the *insets*. Standard errors are less than 5% for each point.

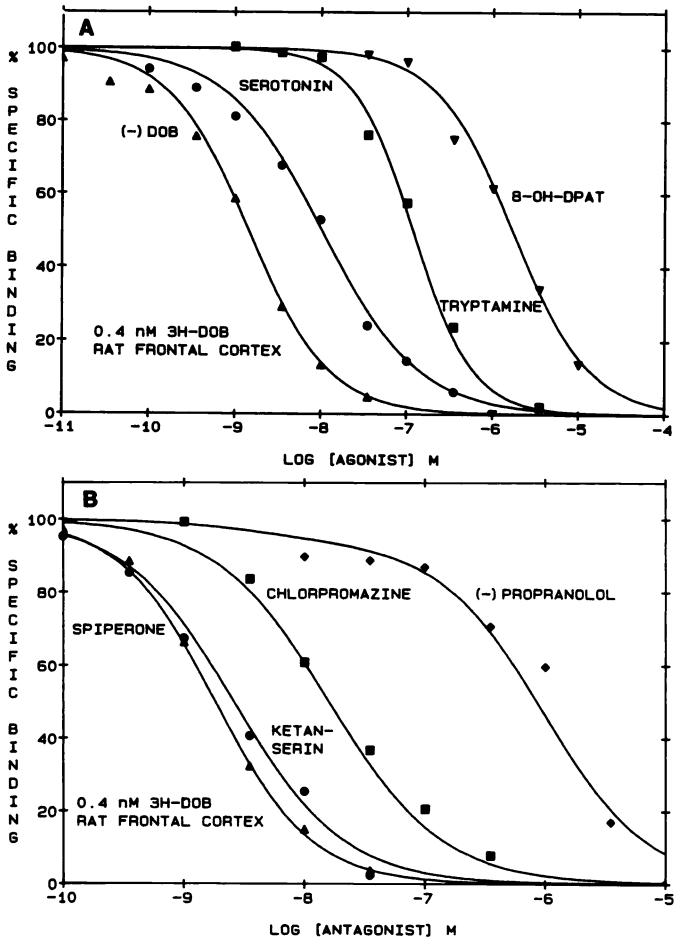

Fig. 4. Agonist (A) and antagonist (B) competition experiments for ^{3}H-DOB binding. Computer-generated curves are the best fit of the binding data. Standard errors are less than 5% for each point.

ing cortical 5-HT$_2$ receptors. The 5-HT$_2$ receptor antagonists cinanserin, ketanserin, spiperone, and chlorpromazine all compete potently for specific ^{3}H-DOB binding. The competition curves exhibited Hill coefficients close to unity, indicating the likelihood that the majority of the specific binding is to one site. The agonists serotonin, 5-methoxytryptamine, tryptamine, bufotenin, DOB, and quipazine compete potently and also produce Hill coefficients generally close to unity. The

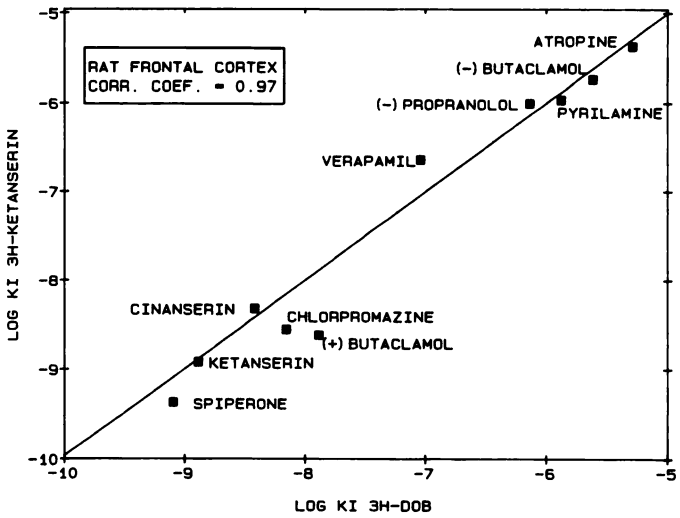

Fig. 5. Correlation of antagonist K_i values for ^{3}H-DOB and ^{3}H-ketanserin specific binding. K_i values were obtained from IC_{50} values according to the Cheng-Prusoff equation (17). Correlation coefficient equals 0.96.

affinities of agonists for ^{3}H-DOB-labeled sites are generally in the same range as agonists for an agonist high affinity state of ^{3}H-ketanserin-labeled 5-HT$_2$ receptors detected in previous studies (5, 6).

The B_{max} for specific ^{3}H-DOB binding is approximately 5% of the B_{max} for the antagonist radioligand ^{3}H-ketanserin. Relatively low levels of ^{3}H-DOB specific binding relative to ^{3}H-ketanserin binding are found throughout the brain regions examined (Table 1). It is not unusual for an agonist radioligand to label a fraction of the sites labeled by an antagonist radioligand (11, 12). It is generally presumed that the radioactive agonist—labeled sites represent receptor/N subunit complexes and that radioactive antagonists label both free receptors and receptor/N subunit complexes. Presumably, ^{3}H-DOB labels a 5-HT$_2$ receptor/N subunit complex. There is evidence from previous radiolabeling studies that 5-HT$_2$ receptors interact with N subunits. Guanyl nucleotides, divalent cations, monovalent cations, and pH, all of which have been shown to regulate receptor/N subunit interactions, have been shown to regulate the agonist-binding properties of ^{3}H-ketanserin-labeled 5-HT$_2$ receptors (4–7). Conn and Sanders-Bush (18, 19) and Kendall and Nahorski (20) have demonstrated that phosphatidylinositol metabolism is stimulated by serotonin through 5-HT$_2$ receptors in cortical slices. Several groups have demonstrated that receptor-mediated stimulation of phosphatidylinositol metabolism involves a N subunit (21–23).

Agonist radioligand binding to receptor/N subunit complexes is sensitive to guanyl nucleotides (11, 12). Presumably, the guanyl nucleotide binds to the N subunit dissociating the complex. As shown in Fig. 6, ^{3}H-DOB binding to the 5-HT$_2$ receptor is sensitive to the nonhydrolyzable guanyl nucleotides GppNHp and GTPγS. The specificity of this inhibition is indicated by the lack of any effect by the adenyl derivatives AppNHp and ATPγS. GTP and GDP gave inconsistent results (data not shown). Substitution of $CaCl_2$ for magnesium in the assay buffer had no effect on GTP and GDP inhibition of ^{3}H-DOB binding (data not shown). Metabolic studies using ^{3}H-GTP indicate that at least 70% of added GTP is metabolized to guanosine in this preparation.[2] This finding undoubtedly

[2] R. A. Lyon, K. H. Davis, and M. Titeler, unpubished data.

TABLE 3

Competition experiments for antagonist- and agonist-radiolabeled 5-HT$_2$ receptors

Reported values are the mean and standard error for three independent experiments performed in triplicate. Eleven concentrations of competing drug were used. K_i values obtained using the Cheng-Prusoff equation (17). Antagonist correlation coefficient equals 0.96. Correlation of ^{3}H-DOB agonist K_i values and ^{3}H-ketanserin agonist high affinity K_i values (Refs. 5 and 8) equals 0.92.

	^{3}H-Ketanserin		^{3}H-DOB	
	K_i	Hill	K_i	Hill
	nM		nM	
5-HT$_2$ agonists				
(−)-DOB	24 ± 3	0.80 ± 0.03	0.39 ± 0.11	0.90 ± 0.11
(±)-DOB	41 ± 5	0.85 ± 0.04	0.79 ± 0.01	0.90 ± 0.02
(+)-DOB	146 ± 9	0.87 ± 0.02	2.3 ± 0.2	0.97 ± 0.02
TFMPP[a]	161 ± 4	0.73 ± 0.01	16.2 ± 1	1.10 ± 0.20
Quipazine	228 ± 8	0.74 ± 0.03	16.8 ± 2	1.12 ± 0.09
5-OH-DMT	297 ± 39	0.71 ± 0.05	6.4 ± 1	1.03 ± 0.12
5-OMe-Tryptamine	305 ± 20	0.75 ± 0.02	4.8 ± 1	0.77 ± 0.13
5-OMe-DMT	616 ± 37	0.85 ± 0.03	15 ± 3	0.81 ± 0.09
RU-24969	777 ± 50	0.86 ± 0.06	42 ± 10	0.87 ± 0.12
Serotonin	928 ± 67	0.70 ± 0.02	7.8 ± 0.8	0.88 ± 0.10
DMT	1,183 ± 36	0.86 ± 0.02	64 ± 10	0.84 ± 0.06
Tryptamine	2,005 ± 116	0.81 ± 0.04	48 ± 5	1.09 ± 0.07
5-HT$_2$ antagonists				
Spiperone	0.42 ± 0.04	0.81 ± 0.05	0.8 ± 0.04	1.12 ± 0.09
Ketanserin	1.2 ± 0.04	1.00 ± 0.07	1.3 ± 0.2	1.05 ± 0.13
(+)-Butaclamol	2.4 ± 0.05	0.88 ± 0.01	13 ± 2	0.88 ± 0.06
Chlorpromazine	2.8 ± 0.03	1.00 ± 0.01	7 ± 1.7	0.92 ± 0.09
Cinanserin	4.8 ± 0.03	0.84 ± 0.04	3.8 ± 0.8	1.13 ± 0.10
(−)-Butaclamol	1,831 ± 71	0.99 ± 0.02	2428 ± 265	0.86 ± 0.06
Non-5-HT$_2$ compounds				
Norepinephrine	>25,000	ND[b]	>25,000	ND
Dopamine	>10,000	ND	>10,000	ND
Histamine	>10,000	ND	>10,000	ND
Prazosin	>10,000	ND	>10,000	ND
Lupitidine	>10,000	ND	>10,000	ND
Pirenzipine	>10,000	ND	>10,000	ND
Nitrendipine	>10,000	ND	>10,000	ND
Verapamil	228 ± 29	1.13 ± 0.02	91 ± 6	1.18 ± 0.12
(−)-Propranolol	989 ± 51	1.09 ± 0.02	730 ± 91	1.37 ± 0.08
Pyrilamine	1,077 ± 48	0.97 ± 0.05	1,324 ± 309	0.88 ± 0.12
(+)-Propranolol	2,490 ± 339	0.91 ± 0.09	1,301 ± 78	0.91 ± 0.06
Atropine	4,257 ± 337	1.10 ± 0.05	5,142 ± 694	1.21 ± 0.11
8-OH-DPAT	5,350 ± 458	0.83 ± 0.07	633 ± 112	0.94 ± 0.04

[a] Abbreviations: TFMPP, trifluoromethylphenylpiperazine; DMT, dimethyltryptamine; 5-OMe, 5-methoxydimethyltryptamine.
[b] ND, not determined.

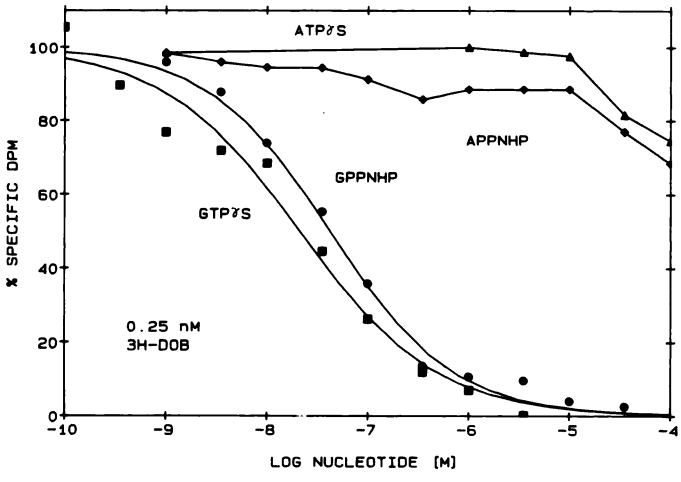

Fig. 6. Guanyl and adenyl nucleotide inhibition of ^{3}H-DOB specific binding. Computer-generated curves are the best fit of the binding data. Standard errors are less than 5% for each point.

TABLE 4

Guanyl and adenyl nucleotide inhibition of ^{3}H-DOB specific binding

Eleven concentrations of nucleotide were used in three independent experiments performed in triplicate. GppNHp and GTPγS inhibited 70% of specific ^{3}H-DOB binding. Values reported are the means and standard errors.

Compound	IC_{50}	Hill
	nM	
GppNHp	41.8 ± 6.1	0.64 ± 0.09
GTPγS	21.4 ± 5.9	0.71 ± 0.05
AppNHp	>10,000	ND[a]
ATPγS	>10,000	ND
ATP	>10,000	ND
ADP	>10,000	ND
AMP	>10,000	ND

[a] ND, not determined.

explains the ineffectiveness of GTP and GDP in inhibiting specific ^{3}H-DOB binding.

Results of previous studies indicated the presence of an agonist high affinity state of the ^{3}H-ketanserin-labeled 5-HT$_2$ receptor (5, 6). The results presented herein indicate that ^{3}H-DOB labels this high affinity state of the 5-HT$_2$ receptor. It is important to note that agonist affinities for the 5-HT$_2$ receptor appear to be 10–100-fold higher in competition studies using ^{3}H-DOB as the radioligand rather than ^{3}H-ketanserin (Table 2). This has important implications in attempting to utilize radioligand assays to determine the 5-HT$_1$ and 5-HT$_2$ receptor specificity of putative serotonergic agents (8). Often, ^{3}H-serotonin, ^{3}H-8-OH-DPAT, or another agonist radioligand has been

used to label 5-HT$_1$ receptors, and either ^{3}H-spiperone or ^{3}H-ketanserin (antagonist radioligands) has been used to label the 5-HT$_2$ receptor (3, 24). The results presented herein clearly illustrate how the apparent affinities of putative agonists for the 5-HT$_2$ receptor may appear different, depending upon whether an agonist radioligand or antagonist radioligand is used. As long as the ligands-of-choice for 5-HT$_1$ receptors are agonist radioligands, it appears that an agonist radioligand for the 5-HT$_2$ receptor, such as ^{3}H-DOB, will more accurately reveal the relative selectivities of putative agonists for the 5-HT$_1$ and 5-HT$_2$ receptors.

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