The Journal of Neuroscience, October 1989, 9(10): 3482–3490

Differentiation of 5-Hydroxytryptamine$_2$ Receptor Subtypes Using ^{125}I-R-(−)2,5-Dimethoxy-4-iodo-phenylisopropylamine and ^{3}H-Ketanserin

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The radioligand binding characteristics of ^{125}I-R-(−)4-iodo-2,5-dimethoxyphenylisopropylamine [^{125}I-R-(−)DOI] and ^{3}H-ketanserin were compared in rat and bovine cortical membranes. In rat cortex, ^{125}I-R-(−)DOI labels a relatively low density of binding sites (B_{max} = 2.5 ± 0.2 pmol/gm tissue) with high affinity (K_D = 0.63 ± 0.09 nM). In bovine cortex, specific binding of ^{125}I-R-(−)DOI represents less than 20% of total binding at radioligand concentrations above 0.6 nM, and, therefore, the data cannot be analyzed adequately by Scatchard transformation. By contrast, ^{3}H-ketanserin displays saturable, specific high-affinity binding in both rat cortex (K_D = 1.0 ± 0.1 nM; B_{max} = 11 ± 0.4 pmol/gm tissue) and bovine cortex (K_D = 1.2 ± 0.2 nM; B_{max} = 5.3 ± 0.4 pmol/gm tissue).

K_i values for 30 drugs were determined for ^{125}I-R-(−)DOI-labeled sites in rat cortex and ^{3}H-ketanserin-labeled sites in bovine cortex. 5-Hydroxytryptamine (5-HT) displays 250-fold higher selectivity for the ^{125}I-R-(−)DOI-labeled sites (K_i = 3.0 ± 0.7 nM) than for the ^{3}H-ketanserin-labeled sites (K_i = 750 ± 50 nM). Structural congeners of R-(−)DOI display 80- to 160-fold higher affinity for the ^{125}I-R-(−)DOI binding site than for the ^{3}H-ketanserin-labeled binding site. *d*-LSD and putative 5-HT$_2$ antagonists are approximately equipotent at both sites. Significant correlations were found between drug affinities for ^{125}I-R-(−)DOI-labeled sites in rat cortex and putative 5-HT$_{2A}$ sites labeled previously by ^{77}Br-R-(−)DOB (r = 0.93, $p < 0.01$), putative 5-HT$_{2B}$ sites labeled by ^{3}H-ketanserin in bovine cortex (r = 0.63, $p < 0.01$), and 5-HT$_{1C}$ binding sites that have been characterized by other investigators (r = 0.78, $p < 0.01$). No significant correlations were found between drug affinities for ^{125}I-R-(−)DOI-labeled sites in rat cortex and 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, or 5-HT$_3$ sites, as determined by previous investigators. We conclude that ^{125}I-R-(−)DOI labels a novel 5-HT binding site subtype (tentatively designated the 5-HT$_{2A}$ binding site) that is present in rat cortex but is either absent or minimally present in bovine cortex. By contrast, ^{3}H-ketanserin labels both the putative 5-HT$_{2A}$ site in rat cortex as well as a separate, distinct recognition site that is present in both rat and bovine cortex, tentatively designated the 5-HT$_{2B}$ site.

Received Jan. 3, 1989; revised Mar. 20, 1989; accepted Mar. 28, 1989.

We thank Debra Bell for her assistance in the preparation of the manuscript. We thank Anne Hamik for excellent technical assistance. This work was supported in part by the McKnight Foundation, the Alfred P. Sloan Foundation, and National Institutes of Health grants NS 12151-14 and NS 23560-03.

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Although 5-hydroxytryptamine (5-HT) has been recognized as a neurotransmitter in the CNS for over 35 years, its physiological roles remain poorly understood. 5-HT appears to play a significant role in a number of human activities such as sleep, appetite, and sexual function (Fuller, 1984; Glennon, 1988; Peroutka, 1988). 5-HT has also been implicated in numerous pathological conditions, including depression (Ogren et al., 1979), migraine (Fozard, 1985; Peroutka, 1988), and Alzheimer's disease (Cross et al., 1984), although a specific pathophysiological dysfunction involving 5-HT has yet to be identified. Conceivably, this failure to identify a clear pathophysiological role for 5-HT relates to the fact that multiple 5-HT receptors exist in the CNS.

The 5-HT$_2$ receptor was first characterized on the basis of its high affinity for a variety of pharmacological antagonists in radioligand binding assays (Leysen et al., 1978; Peroutka and Snyder, 1979; Peroutka et al., 1981; Hoyer et al., 1985; Peroutka, 1988). In the CNS, 5-HT$_2$ antagonists selectively block a slow depolarization induced by 5-HT in both facial motor neurons (McCall and Aghajanian, 1980) and cortical pyramidal neurons (Davies et al., 1987). 5-HT$_2$ receptors also appear to modulate specific 5-HT-induced behavioral responses such as the "head twitch" component of the 5-HT behavioral syndrome and blockade of tryptamine-induced seizures (Leysen et al., 1978, 1984; Peroutka et al., 1981). The discriminative stimulus properties of 5-HT agonists, including a number of hallucinogens, are potently and selectively blocked by 5-HT$_2$ antagonists (Glennon et al., 1984, 1986a). Acute or chronic treatment with various antidepressants, 5-HT agonists and antagonists, 5-HT uptake blockers, and hallucinogens down-regulates 5-HT$_2$ receptors while having relatively little effect on the 5-HT$_1$ class of receptors (Savage et al., 1979; Segawa et al., 1979; Blackshear et al., 1986; Buckholtz et al., 1988; McKenna et al., 1989b). Peripherally, 5-HT$_2$ receptors appear to mediate 5-HT-induced contractions in a number of vascular tissues (Peroutka, 1984), guinea pig ileum (Engel et al., 1984), and tracheal and bronchial smooth muscle (Leysen et al., 1984). 5-HT$_2$ receptors may also mediate regulation of aldosterone production (Matsuoka et al., 1985) and 5-HT-induced platelet aggregation (DeClerck et al., 1983).

Phenylalkylamines such as 4-bromo-2,5-dimethoxyphenylisopropylamine (DOB) and 4-iodo-2,5-dimethoxyphenylisopropylamine (DOI) represent a class of agents that interact potently with 5-HT$_2$ receptors (Shannon et al., 1984; Glennon et al., 1986b). These agents are also potent hallucinogens in humans (Shulgin, 1978). As radioligands, these agents have been hypothesized to label a subset of 5-HT$_2$ receptors in the CNS (Titeler et al., 1985, 1987; Lyon et al., 1987; Glennon et al., 1988). For example, (±)^{3}H-DOB has been suggested to label a

Table 1. Scatchard analysis of ^{125}I-R-(−)DOI and ^{3}H-ketanserin binding sites in rat and bovine cortex

	B_{max} (pmol/gm tissue)	K_D (nM)
Rat cortex		
^{125}I-R-(−)DOI	2.5 ± 0.2	0.63 ± 0.09
^{3}H-ketanserin	11 ± 0.4	1.0 ± 0.1
Bovine cortex		
^{125}I-R-(−)DOI	n.d.	n.d.
^{3}H-ketanserin	5.3 ± 0.4	1.2 ± 0.2

Saturation analyses (n = 3) were performed as described in Materials and Methods. Nonspecific binding for ^{3}H-ketanserin was defined with 10^{-6} M cinanserin. Nonspecific binding for ^{125}I-R-(−)DOI was defined with 10^{-7} M 5-HT.
n.d., not detected.

high-affinity "state" of the 5-HT$_2$ receptor (Titeler et al., 1985; Lyon et al., 1987). ^{125}-I-DOI is another phenylalkylamine radioligand that has been analyzed in both autoradiographic (McKenna and Saavedra, 1987; McKenna et al., 1987, 1989a) and homogenate binding studies (Johnson et al., 1987; Glennon et al., 1988) and has also been suggested to label a high-affinity state of the 5-HT$_2$ recognition site. Initially, our laboratory used ^{77}Br-R-(−)DOB (Peroutka et al., 1988; Wang et al., 1988) to label a high-affinity phenylalkylamine recognition site. We have suggested that radioligands such as ^{77}Br-R-(−)DOB and (±)^{3}H-DOB label a distinct subtype, as opposed to state, of the 5-HT$_2$ receptor, tentatively designated the 5-HT$_{2A}$ recognition site (Pierce and Peroutka, 1989). Since an extensive characterization of ^{125}I-DOI binding in brain homogenates has not yet been reported, we conducted a detailed comparison of the radioligand binding properties of ^{125}I-R-(−)DOI with the 5-HT$_2$ antagonist ^{3}H-ketanserin.

Materials and Methods

Radioligand binding assays were performed according to the methods of Peroutka (1986). Briefly, frozen rat brains or portions of bovine brain, obtained originally from frontal-parietal regions of fresh bovine brains, were thawed from −70°C and cortical tissue was dissected. Tissues were homogenized in 20 vol of 50 mM Tris-HCl (pH 7.7, 25°C) and centrifuged at 45,000 × g for 10 min. The pellet was resuspended in Tris-HCl buffer and incubated for 10 min at 37°C. After a second centrifugation, the pellet was resuspended in 80 vol of a buffer consisting of 50 mM Tris-HCl, 4 mM calcium chloride, 0.1% ascorbate, and 10^{-5} M pargyline. The homogenates were immediately used in the binding assay.

Assays consisted of 0.1 ml of a solution of ^{125}I-R-(−)DOI or 0.1 ml of a solution of ^{3}H-ketanserin (New England Nuclear), 0.8 ml of tissue suspension, and 0.1 ml of buffer or displacing drug. Specific activities of the radioligands were 1800 Ci/mmol for ^{125}I-R-(−)DOI (corrected for decay on the day of the experiment) and 61.8 Ci/mmol for ^{3}H-ketanserin. The ^{125}I-R-(−)DOI used in these experiments was generously provided by Dr. C. A. Mathis (Lawrence Berkeley Laboratory); the radiosynthesis and procedures for determining the specific activity of this radioligand have been described elsewhere (Mathis et al., 1988). For saturation experiments, ^{125}I-R-(−)DOI was diluted 1:10 with cold carrier R-(−)DOI. Saturation binding was determined in triplicate over a concentration range of 0.025–5.5 nM for both ^{125}I-R-(−)DOI and ^{3}H-ketanserin. Nonspecific binding was defined for ^{125}I-R-(−)DOI as the excess over blanks taken in the presence of 10^{-7} M 5-HT, while for ^{3}H-ketanserin, 10^{-6} M cinanserin was used to define nonspecific binding. Drugs were dissolved in buffer, except for (+)butaclamol, which was dissolved to 10^{-3} M in ethanol and then diluted in buffer. Following incubation at 25°C for 30 min, the assay mixtures were rapidly filtered through #32 glass fiber filters (Schleicher and Schuell, Keene, NH) and washed twice with 5 ml of 50 mM Tris-HCl buffer. Radioactivity for ^{3}H-ketanserin binding was measured by liquid scintillation spectroscopy

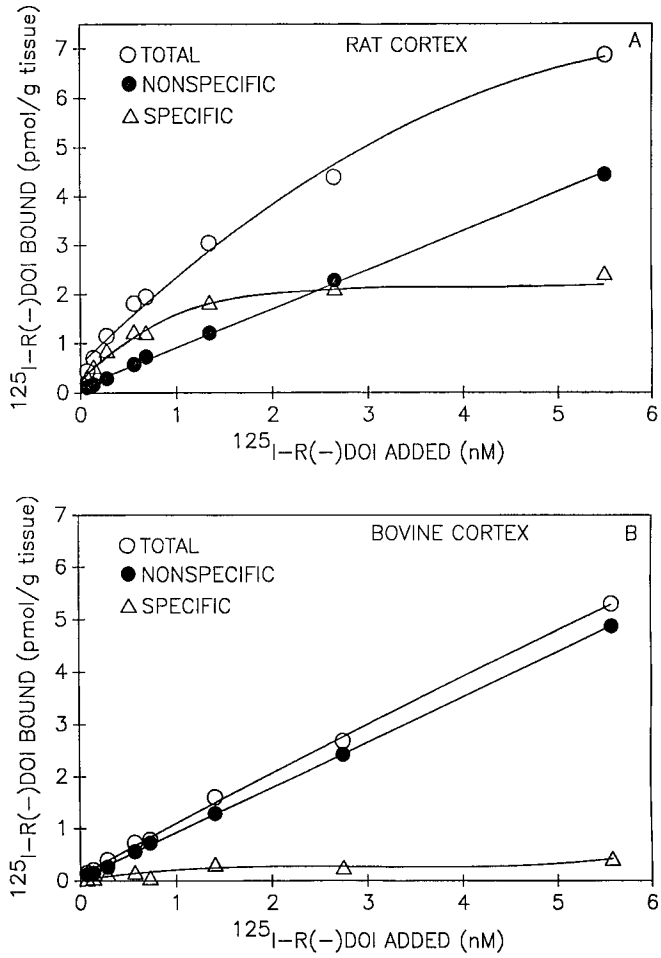

Figure 1. Saturation analysis of ^{125}I-R-(−)DOI binding in rat and bovine brain. Radioligand binding assays were performed as described in Materials and Methods (n = 3). Data given are the results of a single, representative experiment, performed in triplicate: *A*, rat cortical membranes; *B*, bovine cortical membranes.

in 2 ml of Aquasol (New England Nuclear, Boston, MA) at an estimated 65% efficiency. ^{125}I-R-(−)DOI binding was measured in a Searle model 1190 gamma counter at an estimated efficiency of 78%. Competition experiments using ^{125}I-R-(−)DOI were performed using 0.02–0.1 nM of radioactive ligand. At these concentrations, 60–70% of the total binding was specific. Competition experiments with ^{3}H-ketanserin used 0.1–0.2 nM radioactive ligand; specific binding was 75–85% of total binding.

Drugs were obtained from commercial sources except for the following: (+)DOB, (−)DOB, *d*-LSD, (−)DOET, psilocybin, psilocin, (±)3,4,5-TMA, (±)-2,5-DMA, and (−)DOM (National Institute on Drug Abuse, Bethesda, MD); 5-carboxyamidotryptamine (5-CT) (generous gift of Dr. Roger Whiting of Syntex Corporation, Palo Alto, CA); S-(+)DOI and R-(−)DOI (generously provided by Dr. D. E. Nichols, Department of Medicinal Chemistry and Pharmacognosy, Purdue University).

Results

Saturation analysis of ^{125}I-R-(−)DOI and ^{3}H-ketanserin binding in rat and bovine cortical membranes

In rat cortical membranes, ^{125}I-R-(−)DOI displays saturable, high-affinity specific binding. For example, in the single experiment shown in Figure 1*A*, ^{125}I-R-(−)DOI displays half-maximal specific binding at approximately 0.5 nM. At this concentration of radioligand, specific binding represents approximately 70% of total binding. At the highest concentration of ^{125}I-R-(−)DOI used in this experiment (5.5 nM), specific binding represents 35% of total binding. By contrast, a strikingly different

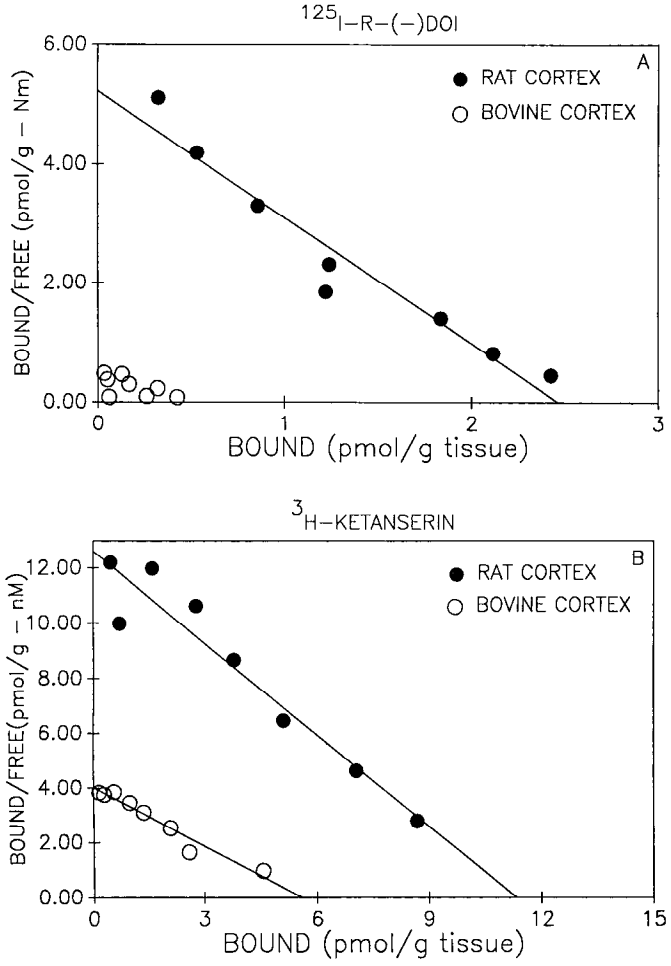

Figure 2. Scatchard analysis of saturation data for ^{125}I-R(−)DOI and ^{3}H-ketanserin in rat and bovine cortical membranes. Radioligand binding assays were performed as described in Materials and Methods (n = 3). Data given are the results of a single, representative experiment, performed in triplicate: *A*, ^{125}I-R-(−)DOI; *B*, ^{3}H-ketanserin.

pattern is observed with ^{125}I-R-(−)DOI binding in bovine cortical membranes. In this tissue, saturable, specific binding of ^{125}I-R-(−)DOI cannot be detected (Fig. 1*B*). Specific binding represents less than 20% of total binding at ^{125}I-R-(−)DOI concentrations above 0.6 nM. Statistically significant specific ^{125}I-R-(−)DOI binding cannot be detected in the bovine cortex at radioligand concentrations above 4 nM, where specific binding represents only 8 ± 1% of total radioligand binding (n = 3).

The Scatchard plots of these data, analyzed according to the method of Rosenthal (1967), are shown in Figure 2*A*. In rat cortical membranes, Scatchard analysis of the saturation data indicates a K_D of 0.63 nM for ^{125}I-R-(−)DOI with a B_{max} value of 2.5 ± 0.2 pmol/gm tissue. The binding data for ^{125}I-R-(−)DOI in bovine cortex cannot be analyzed using the Scatchard transformation due to the extremely low level of specific binding, relative to total binding, that is detected in this tissue. A summary of all saturation experiments is provided in Table 1.

By contrast, specific and saturable ^{3}H-ketanserin binding can be detected in both rat and bovine brain membranes (Fig. 2*B*). In rat cortical membranes, ^{3}H-ketanserin displays an apparent K_D of 1.0 ± 0.1 nM and a B_{max} of 11 ± 0.4 pmol/gm tissue (Table 1). In bovine cortex, ^{3}H-ketanserin displays an apparent

Table 2. Species distribution of putative 5-HT$_{2A}$ binding sites labeled by 0.1 nM ^{125}I-R-(−)DOI

Species	Specific binding (pmol/gm tissue)	Percent of rat cortex
Mouse	0.35 ± 0.02	130
Dog	0.32 ± 0.07	118
Rat	0.27 ± 0.03	100
Human	0.24 ± 0.09	89
Pig	0.24 ± 0.04	89
Rabbit	0.19 ± 0.03	70
Chicken	0.19 ± 0.03	70
Guinea pig	0.19 ± 0.04	70
Bovine	0.07 ± 0.004	26

Radioligand binding studies using 0.1 nM ^{125}I-R-(−)DOI were performed as described in Materials and Methods. Specific binding was defined as the difference between total pmol bound/gm tissue and pmol bound/gm tissue in the presence of 10^{-7} M 5-HT. Values given are means ± SE of 3–5 separate experiments, with each experiment performed in triplicate.

K_D of 1.2 ± 0.2 nM. However, the B_{max} for ^{3}H-ketanserin in bovine membranes is 5.3 ± 0.4 pmol/gm tissue, approximately 2-fold less than the binding density in rat cortex (Table 1).

Species distribution of ^{125}I-R-(−)DOI binding sites

Radioligand binding studies were performed with ^{125}I-R-(−)DOI using tissues from a variety of species. As shown in Table 2, considerable variation exists in the amount of specific ^{125}I-R-(−)DOI binding using 0.1 nM radioligand in various mammalian species. Specific binding ranged from more than 100% of the amount observed in rat cortex (i.e., mouse and dog) to less than 30% (i.e., bovine cortex). The phylogenetic and/or functional significance of the species variations is unclear at the present time.

Drug competition studies with ^{125}I-R-(−)DOI- and ^{3}H-ketanserin-labeled binding sites in rat and bovine cortex

Drug competition studies were performed using ^{125}I-R-(−)DOI in rat cortex and ^{3}H-ketanserin in bovine cortex (Table 3). 5-HT is the most selective agent in terms of its relative affinity for the 2 sites. In the rat cortex, 5-HT displays a K_i value of 3.0 ± 0.7 nM and a Hill slope of approximately unity. Using ^{3}H-ketanserin as the radiolabel in rat cortex, the competition curve produced by 5-HT is relatively shallow, with a Hill slope of 0.67. A third pattern of 5-HT competition is observed when ^{3}H-ketanserin is used in bovine cortex. 5-HT appears to be least potent under this condition, with 10^{-7} M 5-HT displacing only 20–30% of specific ^{3}H-ketanserin binding. The apparent K_i value of 5-HT versus ^{3}H-ketanserin in bovine cortex is 750 ± 50 nM. Importantly, the Hill slope of 5-HT versus ^{3}H-ketanserin binding in the bovine cortex is 0.96. Therefore, 5-HT displays approximately 250-fold higher potency for ^{125}I-R-(−)DOI-labeled sites than for ^{3}H-ketanserin-labeled sites (Table 3, Fig. 3).

The most potent competitors for the sites labeled by ^{125}I-R-(−)DOI are phenylalkylamines, i.e., 4-substituted 2,5-dimethoxyphenylisopropylamines such as (−)DOB, (−)DOM, and (−)DOI itself. These agents display subnanomolar affinity for specific ^{125}I-R-(−)DOI binding (Table 3). By contrast, these agents are approximately 100-fold less potent in displacing specific ^{3}H-ketanserin binding in bovine cortex. Indeed, most drugs examined in the competition studies are more potent at ^{125}I-R-

Table 3. Drug affinities for putative 5-HT_{2A} binding sites labeled by ^{125}I-R-(−)DOI in rat cortex and putative 5-HT_{2B} sites labeled by ^{3}H-ketanserin in bovine cortex

Drug	K_i (nM) ^{125}I-DOI (5-HT_{2A})	^{3}H-ketanserin (5-HT_{2B})	Ratio 2B/2A
1. 5-HT	3.0 ± 0.7	750 ± 50	250
2. (−)DOET	0.54 ± 0.1	80 ± 10	150
3. (−)DOB	0.40 ± 0.04	60 ± 5	150
4. Mescaline	150 ± 10	20,000 ± 4000	130
5. (+)DOI	0.89 ± 0.3	100 ± 20	130
6. (+)DOB	1.0 ± 0.1	125 ± 10	130
7. 5-CT	88 ± 10	11,000 ± 1000	130
8. Tryptamine	11 ± 1	1300 ± 40	120
9. (−)DOI	0.53 ± 0.2	55 ± 5	110
10. (±)3,4,5-TMA	73 ± 10	7800 ± 2000	100
11. 5-MeO-DMT	14 ± 4	1300 ± 300	93
12. Quipazine	5.0 ± 0.5	460 ± 20	90
13. (−)DOM	4.8 ± 1.0	400 ± 60	83
14. (±)2,5-DMA	100 ± 6	6800 ± 1000	68
15. RU 24969	19 ± 9	1200 ± 100	63
16. Psilocybin	13 ± 4	600 ± 40	46
17. mCPP	21 ± 5	960 ± 600	46
18. Psilocin	12 ± 3	450 ± 60	38
19. *N,N*-DMT	42 ± 10	550 ± 20	13
20. 8-OH-DPAT	700 ± 200	5400 ± 800	8
21. Mesulergine	3.2 ± 0.7	8.0 ± 1	3
22. *d*-LSD	3.5 ± 0.4	11 ± 3	3
23. Ketanserin	1.2 ± 0.1	2.4 ± 0.1	2
24. Cinanserin	7.0 ± 1	5.0 ± 1	0.7
25. Chlorpromazine	11 ± 0.3	6.0 ± 0.7	0.5
26. Spiperone	1.6 ± 0.3	0.80 ± 0.1	0.5
27. Metergoline	5.0 ± 2	2.3 ± 0.5	0.5
28. (+)Butaclamol	60 ± 20	17 ± 3	0.3
29. ICS-205930	12,000 ± 4000	2600 ± 600	0.2
30. (−)Butaclamol	>10,000	17,000 ± 4000	>0.2

Radioligand binding assays were performed as described in Materials and Methods. IC_{50} values were determined by log-logit analysis. K_i values were calculated from the formula $K_i = IC_{50}/1 + [I]/K_D$ where K_D for ^{125}I-R-(−)DOI was 0.63 nM and $[I]$ ranged from 0.02 to 0.1 nM. The K_D for ^{3}H-ketanserin was 1.2 nM and $[I]$ ranged from 0.1 to 0.2 nM. Values given are the means ± SE of 3–6 experiments, each performed in triplicate.

(−)DOI-labeled sites than at ^{3}H-ketanserin-labeled sites (Table 3).

Multiple 5-HT_2 antagonists display nanomolar affinity for both ^{125}I-R(−)DOI- and ^{3}H-ketanserin-labeled sites (Table 3). Both LSD and spiperone, for example, display approximately equal affinity for both sites. Ketanserin is also equipotent at the 2 sites, a finding consistent with its apparent ability to label 2 distinct recognition sites. No agents were identified which displayed more than an order of magnitude higher affinity for ^{3}H-ketanserin-labeled sites compared with ^{125}I-R(−)DOI-labeled sites.

Correlation of ^{125}I-R-(−)DOI-labeled sites with drug affinities for 5-HT receptor subtypes

Drug affinities for ^{125}I-R-(−)DOI-labeled sites in rat cortex were compared with drug affinities for ^{77}Br-R-(−)DOB-labeled sites determined previously (Peroutka et al., 1988) and with drug affinities for ^{3}H-ketanserin-labeled sites in bovine cortex (Table 3). Correlations were also determined for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, and 5-HT_3 sites as reported by other laboratories. As expected on the basis of their structural similarity, a highly significant correlation is found between drug affinities for sites labeled by ^{125}I-R-(−)DOI with drug affinities for sites labeled by ^{77}Br-R-(−)DOB (Fig. 4*A*; $r = 0.93$, $p < 0.01$; $n = 26$). A lower, but still significant, correlation is found between drug affinities for ^{125}I-R-(−)DOI-labeled sites in rat cortex and ^{3}H-ketanserin-labeled sites in bovine cortex (Fig. 4*B*; $r = 0.63$, $p < 0.01$; $n = 30$). A significant correlation is also found between drug affinities for ^{125}I-R-(−)DOI sites and 5-HT_{1C} sites (Fig. 4*C*; $r = 0.78$, $p < 0.01$; $n = 21$) characterized by other investigators. Therefore, these data are consistent with an *identity* between sites labeled by ^{125}I-R(−)DOI and ^{3}H-(±)DOB or ^{77}Br-R-(−)DOB and a *similarity* between ^{125}I-R-(−)DOI-labeled sites and putative 5-HT_{2B}- or 5-HT_{1C}-binding sites.

As shown in Figure 5, no significant correlations could be detected between drug affinities for ^{125}I-R-(−)DOI-labeled sites and 5-HT_{1A} ($r = 0.41$, $p > 0.05$; $n = 21$), 5-HT_{1B} ($r = 0.06$, $p > 0.05$; $n = 21$), 5-HT_{1D} ($r = 0.06$, $p > 0.05$; $n = 21$), or 5-HT_3 binding sites ($r = 0.49$, $p > 0.05$; $n = 21$) as defined in previous studies.

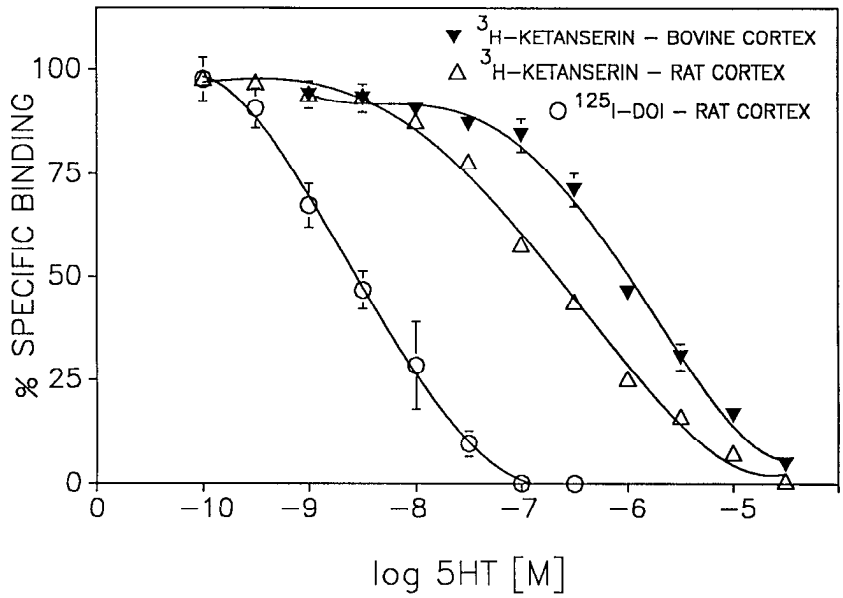

Figure 3. 5-HT competition studies versus ^{125}I-R-(−)DOI binding in rat cortex and ^{3}H-ketanserin in rat and bovine cortex. Radioligand binding assays were performed as described in Materials and Methods. Nonspecific binding for ^{125}I-R-(−)DOI was defined with 10^{-7} M 5-HT, and nonspecific binding for ^{3}H-ketanserin was defined with 10^{-6} M cinanserin. Data given are means ± SE of 6 independent experiments, each performed in triplicate.

Correlation between drug affinities for ^{125}I-R-(−)DOI-labeled sites and human hallucinogenic doses

Drug affinities for ^{125}I-R-(−)DOI-labeled sites were correlated with human hallucinogenic doses as reported in Shulgin (1978) and Glennon and Rosecrans (1982). A highly significant correlation was found (Fig. 6; $r = 0.86$, $p < 0.01$; $n = 14$). Thirteen of the 14 drugs analyzed lie close to the linear-regression line. The single exception is *d*-LSD, which has significantly lower affinity for ^{125}I-R-(−)DOI sites than might be expected on the basis of its human hallucinogenic potency. These data suggest that the sites labeled by ^{125}I-R-(−)DOI may play a role in the pathophysiology of hallucinosis.

Discussion

The major finding of the present study is that ^{125}I-R-(−)DOI labels a saturable, high-affinity population of specific binding sites in rat cortex that are similar, but not identical, to binding sites labeled by ^{3}H-ketanserin in bovine cortex and to 5-HT$_{1C}$ binding sites. In the rat cortex, these sites constitute approximately 20% of the total binding site density labeled by ^{3}H-ketanserin in the same tissue. In bovine cortex, however, a specific, saturable, high-affinity recognition site for ^{125}I-R-(−)DOI cannot be detected. These data indicate that ^{125}I-R-(−)DOI specifically labels a 5-HT recognition site in rat cortex that does not exist, or cannot be detected due to its low density, in bovine brain. These data also support the recent observations of Pierce and Peroutka (1989) using ^{3}H-(±)-DOB in both rat and bovine brain.

The present study provides radioligand binding evidence for the existence of distinct 5-HT$_2$-recognition sites in brain membranes. Furthermore, the presence of these putative 5-HT$_2$ binding site subtypes appears to vary in different mammalian species. In accordance with accepted nomenclature, we propose that the high-affinity site labeled by ^{125}I-R-(−)DOI, ^{3}H-(±)-DOB, and ^{77}Br-R-(−)DOB be designated 5-HT$_{2A}$ membrane recognition sites. ^{3}H-Ketanserin labels both these putative 5-HT$_{2A}$ sites as well as a subpopulation of 5-HT$_2$ recognition sites in bovine brain, which we suggest be designated as 5-HT$_{2B}$ binding sites.

The results of our competition experiments using ^{125}I-R-(−)DOI in rat cortex and ^{3}H-ketanserin in bovine cortex also support the hypothesis of 5-HT$_2$ binding site heterogeneity. 5-HT is the most "selective" agent that can be used to discriminate the 2 sites (Table 3). Specifically, 5-HT displays nanomolar affinity for ^{125}I-R-(−)DOI-labeled sites (K_i = 3.0 ± 0.7 nM), while it displays 250-fold weaker affinity for the sites labeled by ^{3}H-ketanserin (K_i = 750 ± 50 nM). The 4-substituted, 2,5-dimethoxyphenylisopropylamines also discriminate between the 2 sites. Each of these agents shows an approximately 100-fold higher affinity for the 5-HT$_{2A}$ sites labeled by ^{125}I-R-(−)DOI than for the 5-HT$_{2B}$ sites labeled by ^{3}H-ketanserin (Table 3). By contrast, 5-HT$_2$ antagonists show equal affinity for both sites or slightly higher affinity for the 5-HT$_{2B}$ site (Table 3).

Corrclation analyses of drug affinities also support the interpretation that ^{125}I-R-(−)DOI in rat cortex and ^{3}H-ketanserin in bovine cortex are labeling different populations of binding sites (Figs. 4, 5). There is a highly significant correlation between drug affinities for the ^{125}I-R-(−)DOI site and the site labeled by ^{77}Br-R-(−)DOB ($r = 0.93$, $p < 0.01$), strongly suggesting that these radioligands are labeling the same membrane recognition sites (Fig. 4*A*). However, there is also a significant but lower correlation between drug affinities for the ^{125}I-R-(−)DOI site and the ^{3}H-ketanserin labeled site in bovine cortex (Fig. 4*B*; $r = 0.68$, $p < 0.01$). These observations suggest that the "5-HT$_2$" binding site labeled by ^{3}H-ketanserin is similar but not identical to the site labeled by ^{125}I-R-(−)DOI and ^{77}Br-R-(−)DOB. Indeed, most previous studies have been primarily directed at the 5-HT$_{2B}$ receptor while tending to overlook the 5-HT$_{2A}$ receptor. This is not surprising in view of the fact that the 5-HT$_{2A}$ binding component comprises only 15–20% of the total "5-HT$_2$" binding, as measured using ^{3}H-ketanserin in rat cortex.

A significant correlation also exists between drug affinities for the ^{125}I-R-(−)DOI binding site and the 5-HT$_{1C}$ recognition site, as characterized by other investigators (Fig. 4*C*). This observation provides further evidence for the similarities between the 5-HT$_{1C}$ recognition site and the 5-HT$_2$ receptor "family." Hoyer (1988) has commented on the pharmacological and biochemical similarities between the 5-HT$_{1C}$ and 5-HT$_2$ receptors and has suggested that the 5-HT$_{1C}$ receptors should be more appropriately classified as a 5-HT$_2$ receptor subtype. Our data agree with this suggestion.

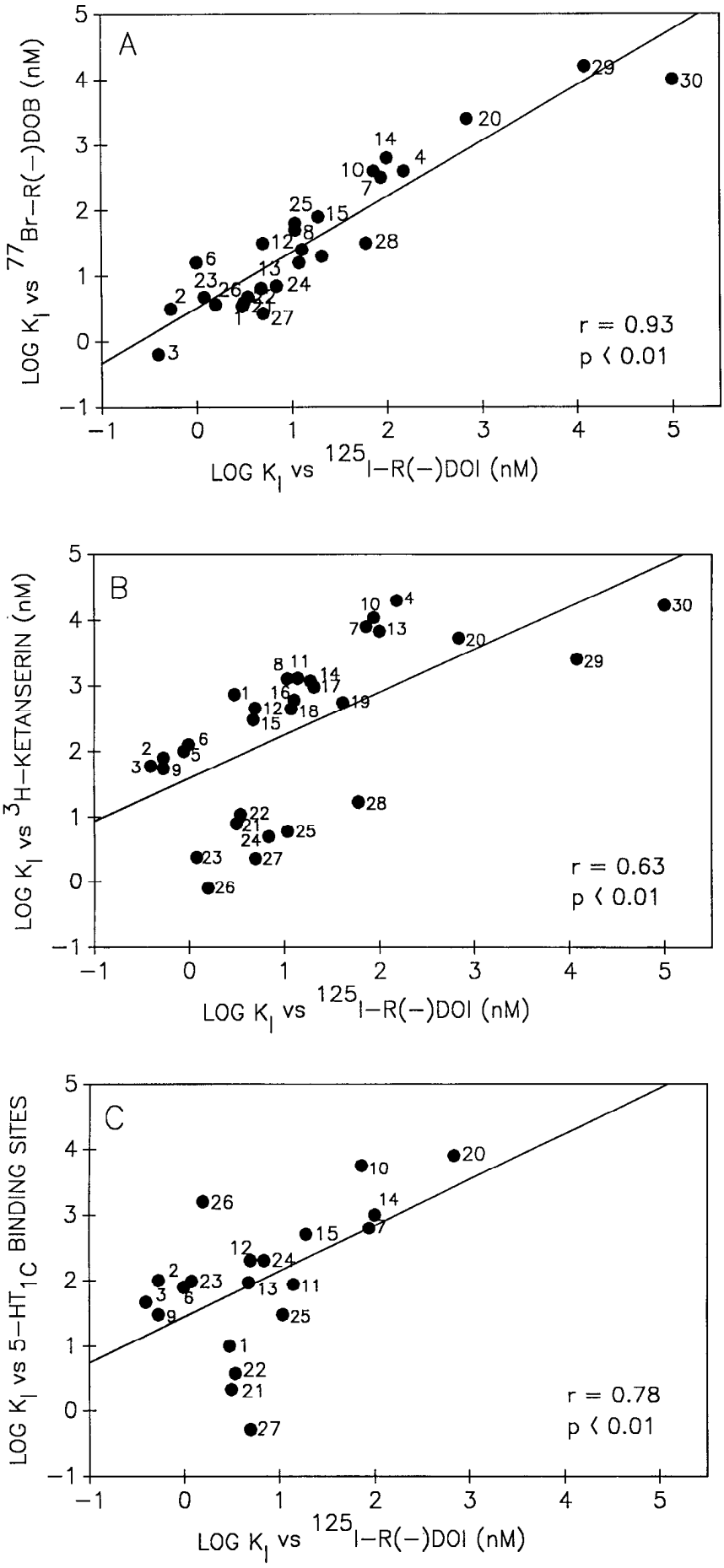

Figure 4. Correlation of drug affinities for ^{125}I-R-(−)DOI-labeled binding sites in rat cortex with other radioligand binding sites. Drug affinities for ^{125}I-R-(−)DOI were obtained from Table 3 and correlated with drug affinities for *A*, ^{77}Br-R-(−)DOB-labeled sites (Peroutka et al., 1988); *B*, ^{3}H-ketanserin-labeled sites in bovine cortex (Table 3); and *C*, 5-HT_{1C} binding sites (Pazos et al., 1984; Hoyer et al., 1985; Peroutka, 1986; Titeler et al., 1988).

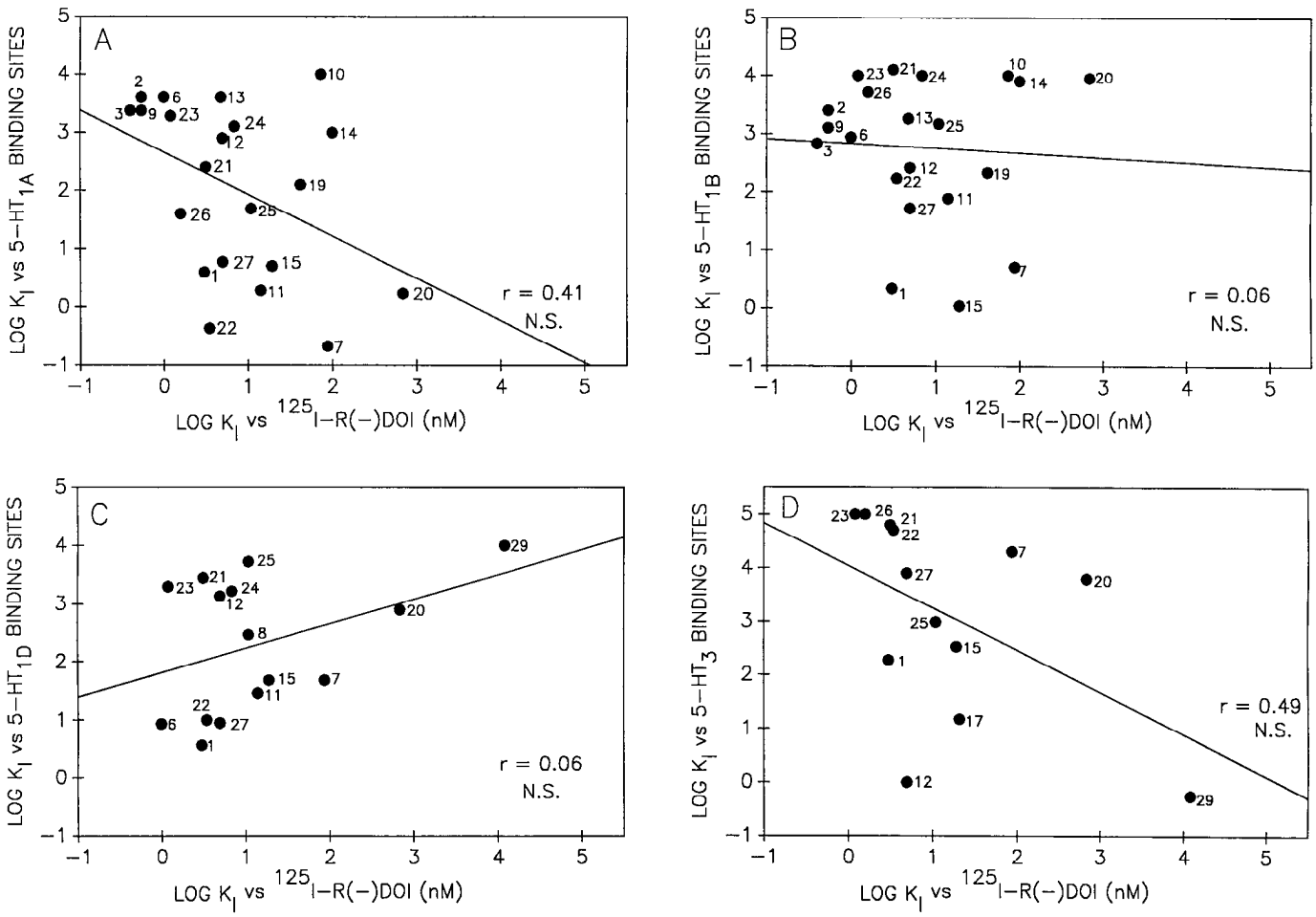

Figure 5. Correlation of drug affinities for ^{125}I-R-(−)DOI-labeled binding sites in rat cortex with other radioligand binding sites. Drug affinities for ^{125}I-R-(−)DOI were obtained from Table 3 and correlated with drug affinities for *A*, 5-HT$_{1A}$ binding sites (Hoyer et al., 1985; Peroutka, 1986; Titeler et al., 1988); *B*, 5-HT$_{1B}$ binding sites (Hoyer et al., 1985; Peroutka, 1986; Titeler et al., 1988); *C*, 5-HT$_{1D}$ binding sites (Hoyer et al., 1988; Herrick-Davis et al., 1988); and *D*, 5-HT$_3$ binding sites (Hoyer and Neijt, 1988; Peroutka and Hamik, 1988).

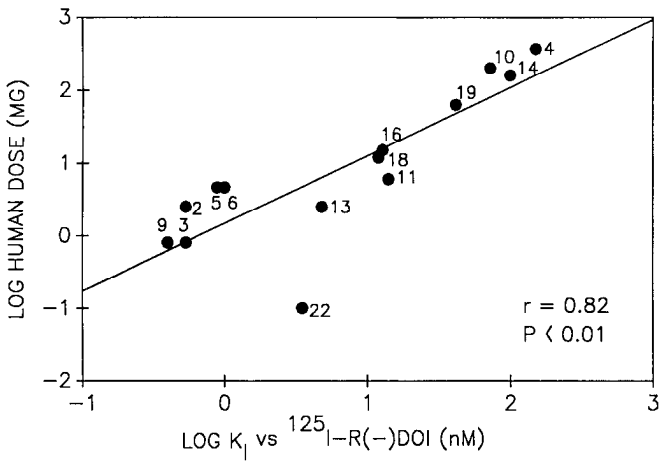

Figure 6. Correlation of drug affinities for ^{125}I-R-(−)DOI-labeled sites in rat cortex with human hallucinogenic doses. Drug affinities for ^{125}I-R-(−)DOI were obtained from Table 3 and correlated with human hallucinogenic drug dosages as reported by Shulgin (1978) and Glennon and Rosecrans (1982).

Alternatively, the apparent differences between ^{125}I-R-(−)DOI binding and ^{3}H-ketanserin binding could be due to the ability of ^{125}I-R-(−)DOI to label 5-HT$_{1C}$ sites as well as 5-HT$_2$ sites in the rat cortex. Indeed, McKenna et al. (1989a) showed that ^{125}I-R-(−)DOI does label 5-HT$_{1C}$ sites in the rat choroid plexus. However, Hoyer (1988) has shown that 5-HT$_{1C}$ receptors are absent or only minimally present in rat cortex. Since all of the studies in the present report were performed in rat cortex, it is unlikely that 5-HT$_{1C}$ binding accounts for a significant portion of the binding sites analyzed. This conclusion is further confirmed by the lack of identity between drug affinities for ^{125}I-R-(−)DOI binding sites and 5-HT$_{1C}$ sites (see Fig. 4*C*).

These data are consistent with the extensive observations of Titeler and colleagues (Battaglia et al., 1984; Titeler et al., 1985, 1987; Lyon et al., 1987; Glennon et al., 1988). These investigators proposed that the agonist radioligand ^{3}H-(±)DOB labels a "high-affinity agonist state" of the 5-HT$_2$ receptor that represents receptor/N subunit complexes, while antagonist radioligands such as ^{3}H-ketanserin label both free receptors and receptor/N subunit complexes. This hypothesis derived, in large part, from the observation that drugs such as 5-HT and the

phenylisopropylamines display low Hill slopes against ^{3}H-ketanserin binding in rat cortex. However, if the "5-HT_2" receptor in bovine and rat brain were identical, then similar experimental results would be expected in both tissues. The present data are clearly inconsistent with this hypothesis. Alternatively, we suggest that the site labeled by ^{125}I-R-(−)DOI (the putative 5-HT_{2A} binding site) is a distinct molecular entity and not a high-affinity agonist "state" of the 5-HT_2 receptor.

Conceivably, our failure to detect a "high-affinity agonist state" of the 5-HT_2 receptor in bovine cortex results from a differential lability of the G-protein associated with the receptor in rat versus bovine brain. For example, differences in the processing of the 2 different tissues (freezing, thawing, etc.) may result in the inability to generate the "high-affinity agonist state" in bovine, as opposed to rat, tissues. This possibility appears unlikely, however, as both rat and bovine tissues were stored under identical conditions and were processed and studied on the same day using identical experimental procedures.

Another important observation is that, in general, 5-HT agonists display higher affinities for the putative 5-HT_{2A} site labeled by ^{125}I-R-(−)DOI than for the 5-HT_{2B} site labeled by ^{3}H-ketanserin. This finding may be viewed as providing general support for the hypothesis that ^{125}I-R-(−)DOI labels a "high-affinity agonist state" of the 5-HT_2 receptor. Therefore, a clear need exists to develop antagonists that preferentially interact with ^{125}I-R-(−)DOI binding sites in order to provide further support for our hypothesis of 2 distinct 5-HT_2 binding site subtypes. Until such agents become available, we do not believe that the "high affinity agonist state" hypothesis of Titeler and colleagues should be completely discarded.

Finally, the structural similarities of DOB and its iodinated congener, DOI, and their similar pharmacological action as human hallucinogens, suggest that both agents interact with identical receptors. The high affinity of many hallucinogens for 5-HT_{2A} receptors (Table 3) and the significant correlation that exists between these affinities and human hallucinogenic doses (Fig. 6) indicate that this receptor subtype may play a role in hallucinosis. Future studies are likely to further define the functional and clinical relevance of the 5-HT_{2A} binding site and should facilitate the understanding of 5-HT in both normal and abnormal brain function.

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