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DIFFERENTIAL INTERACTIONS OF INDOLEALKYLAMINES WITH 5-HYDROXYTRYPTAMINE RECEPTOR SUBTYPES

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Summary—Affinities of drugs for 21 indolealkylamine derivatives, some with putative hallucinogenic activity, were determined at 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2B} recognition sites, using radioligand competition studies. Nearly all of the derivatives displayed greatest potency for the 5-HT_{2A} receptor, labelled by [^{125}I]*R*-(−)DOI in the cortex of the rat. Most derivatives displayed 2–10 times lower affinity at the HT_{2B} receptor labelled by [^{3}H]ketanserin in bovine cortex. Derivatives lacking ring substituents displayed lower affinities for all of the recognition sites, compared to derivatives substituted in the 4- or 5-position of the indole ring. The 4-hydroxylated derivatives displayed 25–380-fold selectivity for the 5-HT_{2A} site, vs the 5-HT_{1A} site, while the 5-substituted derivatives displayed approximately equal potency at the 5-HT_{1A} and 5-HT_{2A} sites. Affinity of all the compounds at the 5-HT_{2B} site was greater than 300 nM. The 6-substituted derivatives displayed greater than micromolar affinities for all of the 5-HT recognition sites examined. The size of the *N*,*N*-dialkyl substituent was a secondary determinant of affinity, with groups larger than *N*,*N*-diisopropyl resulting in a marked reduction in affinity at both the 5-HT_{2A} and 5-HT_{1A} recognition sites. This study demonstrated that hallucinogenic 4-hydroxy-indolealkylamines, like psychotomimetic phenylisopropylamines, bind potently and selectively to the 5-HT_{2A} recognition site, labelled by [^{125}I]*R*-(−)DOI. This provides further evidence indicating that this recently described subtype of the 5-HT_2 receptor may partially mediate the action of hallucinogenic agents.

Key words—indolealkylamines, radioligand binding, serotonin receptors, hallucinogens, psilocin, bufotenine.

Most investigators, interested in characterizing the receptor interactions of hallucinogens, have focussed either on *d*-lysergic acid diethylamide (*d*-LSD), since it is the most potent hallucinogen, or on ring-substituted phenylalkylamines, such as 4-methyl-2,5-dimethoxyphenylisopropylamine (DOM), since extensive structure/activity and human data is available for this class of hallucinogenic agents (Shulgin, 1978, 1982). Several lines of evidence have implicated a putative subtype of 5-hydroxytryptamine receptors, the 5-HT_2 receptor, as a probable site of action for hallucinogens. In particular, 4-bromo-2,5-dimethoxyphenylisopropylamine (DOB) and 4-iodo-2,5-dimethoxyphenylisopropylamine (DOI), halogenated analogs of the hallucinogen DOM, are potent and selective ligands for 5-HT_2 receptors (Shannon, Battaglia, Glennon and Titeler, 1984; Glennon, McKenney, Lyon and Titeler, 1986). Investigations in this laboratory using [^{77}Br]*R*-(−)DOB (Peroutka, Hamik, Harrington, Hoffman, Mathis, Pierce and Wang, 1988) and [^{125}I]*R*-(−)DOI (McKenna and Peroutka, 1989) have led to the postulate that these ligands label a distinct subtype of the 5-HT_2 receptor. In accordance with accepted nomenclature, it was proposed (Pierce and Peroutka, 1989; McKenna and Peroutka, 1989) that the novel 5-HT_2 subtype, labelled by [^{125}I]*R*-(−)DOI, be designated the 5-HT_{2A} receptor, and the "classical" 5-HT_2 receptor should be redefined as the 5-HT_{2B} receptor.

Indolealkylamines, such as *N*,*N*-dimethyltryptamine (DMT) and 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT), are the only known hallucinogenic agents whose endogenous occurrence in mammals, including man, has been confirmed (Christian, Harrison, Quayle, Pagel and Monti, 1977; Smythies, Morin and Brown, 1979; Barker, Monti and Christian, 1981). The receptor affinities and/or selectivities of substituted indolealkylamine hallucinogens have not been extensively investigated using some of the more subtype-selective serotonergic radioligands. Earlier studies of receptor interactions of indolealkylamines utilized nonselective radioligands such as [^{3}H]5-HT or [^{3}H]LSD (Kline, Benington, Morin and Beaton, 1982; Glennon, Jacyno, Young, McKenney and Nelson, 1984) or antagonism at peripheral 5-HT receptors, such as the rat fundus strip, to evaluate structure/activity relationships and putative hallucinogenic activity (Glennon, Liebowitz and Mack, 1978; Glennon, Gessner, Godse and Kline, 1979). As far as is known only two recent studies have used selective radioligands to investigate

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the binding of indolealkylamines to 5-HT receptors. Lyon, Titeler, Seggel and Glennon (1988) studied the binding characteristics of 21 indolealkylamine analogs at 5-HT_2 receptors labelled with [^{3}H]ketanserin and reported similarities to the interactions of phenylalkylamine analogs; they also reported a 10- to 100-fold greater potency of selected derivatives, competing at sites labelled with the 5-HT_2 agonist radioligand [^{3}H](±)DOB, in comparison with the antagonist [^{3}H]ketanserin. Engel, Gothert, Hoyer, Schlicker and Hillenbrand (1986) studied the competitive binding of seventeen agonists, including 5-HT and thirteen other indolealkylamines and 13 antagonists at 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_2 receptors. They reported significant correlations between the affinities of the agonists at 5-HT_{1A} and 5-HT_{1B} receptors and pEC_{30}, the inhibition of electrically-evoked release of [^{3}H]5-HT from superfused slices of brain.

Since there has been little effort to determine systematically the influence of ring and *N,N*-dialkyl substituents on affinity or selectivity at 5-HT receptor subtypes, radioligand competition studies were conducted on 21 indolealkylamines (Fig. 1), some with known or putative hallucinogenic properties, and the affinity for 5-HT_{1A} receptors was compared with their affinity at the two putative subtypes of 5-HT_2 receptors.

METHODS

Radioligand binding assays were performed according to the methods of Peroutka (1986) and McKenna and Peroutka (1989). Briefly, frozen rat brains, or portions of bovine cortex, obtained from the frontal-parietal region of fresh bovine brains and previously frozen, were thawed from −70°C and regions of the brain were dissected. Tissues were homogenized in 20 volumes of 50 mM Tris–HCl (pH 7.7 at 25°C) and centrifuged at 30,000 ***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 volumes of a buffer, consisting of 50 mM Tris–HCl, 4 mM calcium chloride, 0.1% ascorbate and 10^{-5} M pargyline. The homogenates were used immediately in the binding assay.

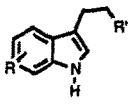
The 5-HT_{1A} sites were labelled with [^{3}H]8-OH-DPAT in the cortex of the rat and nonspecific binding was defined by 10 μM 5-HT (Peroutka, 1986); 5-HT_{2A} sites were labelled in the cortex of the rat with [^{125}I]*R*-(−)DOI, with non-specific binding defined by 10^{-7} M 5-HT (McKenna and Peroutka, 1989); 5-HT_{2B} sites were labelled by [^{3}H]ketanserin in bovine cortex, with non-specific binding defined by 1 μM cinanserin (McKenna and Peroutka, 1989). Assays consisted of 0.1 ml of a solution of either [^{3}H]8-OH-DPAT, [^{3}H]ketanserin (New England Nuclear), or [^{125}I]*R*-(−)DOI, 0.8 ml of tissue suspension and 0.1 ml of buffer or displacing drug. Specific activities of the radioligands were 145 Ci/mmol for [^{3}H]8-OH-DPAT, 61.8 Ci/mmol for [^{3}H]ketanserin or 1700 Ci/mmol for [^{125}I]*R*-(−)DOI (corrected for decay on the day of the experiment). 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, Hoffman, Nichols and Shulgin, 1988). Drugs used in the competition studies were dissolved in buffer, or in 50% ethanol and then diluted in buffer. After incubation at 25°C for 30 min, the assay mixtures were rapidly filtered through No. 32 glass fiber filters (Schleicher and Schuell; Keene, New Hampshire) and washed twice with 5 ml of 50 mM Tris–HCl buffer. Filters were pre-soaked in 0.5% polyethylenimine for the [^{125}I]*R*-(−)DOI assays, but were not pre-soaked for the other ligands. Radioactivity for the tritiated ligand binding was measured by liquid scintillation spectroscopy in 2 ml of Biosafe™ scintillation fluid (Research Products International), at an estimated 41% efficiency. The binding of [^{125}I]*R*-(−)DOI was measured under the same conditions, at an estimated efficiency of 75%. Competition experiments using [^{125}I]*R*-(−)DOI were performed using 0.02–0.05 nM of radioactive ligand. At these concentrations, 70–85% of the total binding was specific. Competition experiments with [^{3}H]ketanserin used 0.1–0.2 nM radioactive ligand; specific binding was 60–70% of total binding. The binding of [^{3}H]8-OH-DPAT used a concentration of 0.2–0.3 nM, with specific binding equal to 80–90% of total binding.

The following drugs were obtained from commercial sources: psilocin (National Institute on Drug Abuse), *N,N*-dimethyltryptamine, 5-hydroxy-*N,N*-dimethyltryptamine and 5-MeO-*N,N*-dimethyltryptamine (Sigma Chemical Co.). The authors are indebted to Dr Leon Lombrozo, Veterans Administration Hospital, Palo Alto, California for providing the sample of 3-[2-(2,5-dimethylpyrrolyl)ethyl]-indole, referred to in Table 1 as *N*-(2,5-dimethyl)-pyrrolyl-tryptamine. Synthesis and chemical characterization of the other tryptamine derivatives used in the assays is described elsewhere (Repke, Grotjahn and Shulgin, 1985; Repke, Ferguson and Bates, 1977, 1981).

The IC_{50} values from competition experiments were determined by log–logit analysis. The IC_{50} values reported in Table 1 are the means, ± standard errors of 3 or more independent determinations, performed in triplicate.

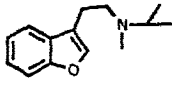
RESULTS

The results of the competition experiments for the 21 tryptamine derivatives are shown in Table 1. Nearly all of the derivatives investigated displayed the greatest potency at the 5-HT_{2A} receptor labelled by [^{125}I]*R*-(−)DOI. By contrast, most derivatives

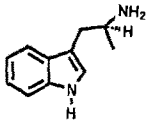


	R	R'
1. 5-OH-N,N-dimethyltryptamine (bufotenine)	5-OH	N-$(CH_3)_2$
2. 5-MeO-N,N-dimethyltryptamine	5-OCH_3	N-$(CH_3)_2$
3. 5-OH-N,N-diisopropyltryptamine	5-OH	N$(iPr)_2$
4. 5-MeO-N-isopropyltryptamine	5-OCH_3	N-iPr
5. 5-MeO-N-methyl,-N-isopropyltryptamine	5-OCH_3	
6. 5-Methyl-N-methyl,-N-isopropyltryptamine	5-CH_3	
7. 4-OH-N,N-dimethyltryptamine (psilocin)	4-OH	N-$(CH_3)_2$
8. 4-OH-N,N-diethyltryptamine (CZ-74)	4-OH	N-$(CH_2CH_3)_2$
9. 4-OH-N-methyl, N-isopropyltryptamine	4-OH	
10. 4-OH-N,N-diisobutyltryptamine	4-OH	
11. 4-OH-N,N-di(sec)butyltryptamine	4-OH	
12. 6-MeO-N-methyl,-N-isopropyltryptamine	6-OCH_3	
13. 5,6-diMeO-N-methyl,-N-isopropyltryptamine	5,6-OCH_3	
14. N,N-dimethyltryptamine	H	N-$(CH_3)_2$
15. N-methyl,-N-isopropyltryptamine	H	

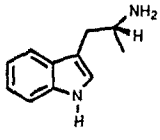
16. N-methyl, N-isopropylbenzofuran



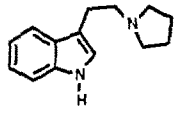
17. S(+)alpha-methyltryptamine



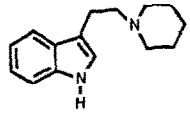
18. R(-)alpha-methyltryptamine



19. N-pyrrolidyltryptamine



20. N-piperidyltryptamine



21. N--2,5-dimethylpyrrolyltryptamine

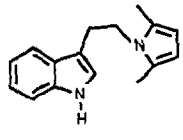


Fig. 1. Structures of indolealkylamine derivatives assayed in radioligand competition studies.

Table 1. Inhibition constants for tryptamine derivatives

Compound and substitution pattern	IC_{50} [nM] 5-HT_{1A}	5-HT_{2A}	5-HT_{2B}
5-Substituted tryptamines:			
1. 5-OH-*N*,*N*-dimethyltryptamine	4.9 ± 0.8	3.5 ± 0.3	370 ± 30
2. 5-MeO-*N*,*N*-dimethyltryptamine	6.5 ± 1.5	14 ± 1	470 ± 30
3. 5-OH-*N*,*N*-diisopropyltryptamine	160 ± 70	5.6 ± 1.4	740 ± 270
4. 5-Meo-*N*-isopropyltryptamine	51 ± 4	50 ± 15	2570 ± 990
5. 5-MeO-*N*-methyl,*N*-isopropyltryptamine	87 ± 14	17 ± 6	1100 ± 170
6. 5-methyl-*N*-methyl,*N*-isopropyltryptamine	290 ± 10	28 ± 6	570 ± 60
4-Substituted tryptamines:			
7. 4-OH-*N*,*N*-dimethyltryptamine	190 ± 40	6.0 ± 0.5	410 ± 50
8. 4-OH-*N*,*N*-diethyltryptamine (CZ-74)	1370 ± 472	14 ± 4	680 ± 50
9. 4-OH-*N*-methyl,*N*-isopropyltryptamine	4900 ± 495	13 ± 4	690 ± 110
10. 4-OH-*N*,*N*-diisobutyltryptamine	6500 ± 1550	260 ± 60	9300 ± 270
11. 4-OH-*N*,*N*-di(sec)butyltryptamine	6470 ± 1340	39 ± 10	1700 ± 290
6-Substituted tryptamines:			
12. 6-MeO-*N*-methyl,*N*-isopropyltryptamine	> 10,000	> 10,000	> 10,000
13. 5,6-diMeO-*N*-methyl,*N*-isopropyltryptamine	> 10,000	5200 ± 2000	> 10,000
Unsubstituted tryptamines:			
14. *N*,*N*-dimethyltryptamine	170 ± 35	75 ± 16	450 ± 24
15. *N*-methyl,*N*-isopropyltryptamine	760 ± 190	38 ± 5	540 ± 50
16. *N*-methyl,*N*-isopropylbenzofuran	5000 ± 1400	500 ± 150	800 ± 125
17. *S*(+)alpha-methyltryptamine	1900 ± 375	46 ± 6	1000 ± 300
18. *R*-(−)alpha-methyltryptamine	1000 ± 240	130 ± 36	3900 ± 820
19. *N*-pyrrolidyltryptamine	30 ± 4	110 ± 20	750 ± 120
20. *N*-piperidyltryptamine	600 ± 90	760 ± 140	1250 ± 300
21. *N*-(2,5-dimethyl)pyrrolyltryptamine	> 10,000	2430 ± 400	> 10,000

were 2–10 times less potent at the 5-HT_{2B} receptor labelled by [^{3}H]ketanserin in bovine cortex. Within each main class of ring-substituted tryptamine derivatives, differential selectivity for each of the receptor subtypes was also observed (Table 1). Hill slopes at each of the recognition sites were determined for psilocin and bufotenine, considered to be representative of the 4- and 5-hydroxylated derivatives, respectively. For psilocin, Hill slope values were 0.83 ± 0.03, 0.82 ± 0.02 and 1.1 ± 0.2 at the 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2B} sites, respectively. Bufotenine gave Hill slope values of 1.1 ± 0.02, 0.79 ± 0.07 and 0.79 ± 0.01 at the 1A, 2A and 2B sites respectively.

4-Hydroxytryptamines

All of the 4-hydroxytryptamines studied were selective for the 5-HT_{2A} site, relative to the other two sites. These derivatives displayed IC_{50} values from 6 to 260 nM at the 5-HT_{2A} site, and from 190 to 6500 nM at the 5-HT_{1A} site. Within the 4-hydroxy compounds, the key parameter determining the rank order of affinity at the 5-HT_{2A} recognition site appeared to be the size of the *N*,*N*-dialkyl substitution, i.e. those compounds substituted with groups larger than isopropyl had a markedly reduced affinity.

5-Substituted tryptamines

Most of the 5-substituted derivatives displayed approximately equal potency for the 5-HT_{1A} and 5-HT_{2A} sites. For example, among the 5-substituted tryptamines, IC_{50} values at the 5-HT_{1A} site ranged from 5 to 290 nM, while at the 5-HT_{2A} site, these values ranged from 3.5 to 50 nM. The size of the *N*,*N*-dialkyl substituent appeared to be a determinant of the rank order of affinity at the 5-HT_{1A} site, but this relationship did not appear to hold at the 5-HT_{2A} site. The polarity and size of the 5-substituent may also affect the affinity, but it is necessary to screen a larger number of compounds before any conclusions can be drawn. 5,6-Disubstituted and 6-substituted derivatives displayed much weaker affinity for all of the receptors screened.

Ring-unsubstituted tryptamines

Ring-unsubstituted and *N*-cycloalkyl tryptamines displayed lower affinities, overall, for all of the recognition sites, compared to derivatives substituted in the 4- or 5-position in the ring. Among the unsubstituted tryptamines, the size or polarity of the *N*,*N*-dialkyl substituent bore no obvious relationship to the rank order of affinity at any of the sites. The difference in affinities between *N*-methyl,*N*-isopropyltryptamine and the benzofuran analog is worth noting; the indole displayed approximately 13- and 7-fold greater affinity at the 5-HT_{2A} and 5-HT_{1A} sites, respectively, than the benzofuran. This indicates that the indolic nitrogen is an important determinant of affinity at these sites. In fact, the importance of the indole nitrogen to the biological activity of the tryptamines has been demonstrated by Winter, Gessner and Godse (1967) and by Pinder, Green and Thompson (1971), in studies in which the indole nitrogen was replaced by oxygen, sulfur or methylene. In a now classical study of tryptamines and receptor interactions, Green, Johnson, Weinstein, Kang and Chou (1978) provided a quantum chemical rationale for the much reduced activity of tryptamine isosteres, relative to their nitrogenous parents. The difference between the benzofuran and tryptamine derivatives at the 5-HT_{2B} site, however, was less marked. Also of interest are the differential selectivities of *R*-(−)alpha-methyltryptamine and

$S(+)$-alpha-methyltryptamine at the 5-HT$_{1A}$ and 5-HT$_{2A}$ sites; while both enantiomers were more potent at the 5-HT$_{2A}$ site, the $R(-)$ enantiomer was only 8-fold more selective, while the $S(+)$ enantiomer was approximately 40-fold more selective for the 5-HT$_{2A}$ site.

DISCUSSION

The major finding of the present study was that 4-hydroxy-*N*,*N*-dialkyltryptamines and some unsubstituted dialkyltryptamines displayed selectivity for the putative 5-HT$_{2A}$ recognition site labelled by [^{125}I]*R*-(−)DOI. Substitution at the 5-position of the indole nucleus enhanced potency, relative to the unsubstituted derivatives, but 5-substituted compounds were approximately equipotent at both the 5-HT$_{1A}$ and 5-HT$_{2A}$ sites. The influence of 4- and 5-hydroxy substitution on the selectivity for the 5-HT subtypes can be more clearly appreciated in Figure 2, which shows competition curves for psilocin and bufotenine, the 4-hydroxy- and 5-hydroxy-isomers, respectively, of *N*,*N*-dimethyltryptamine. Both compounds were approximately equipotent at the 5-HT$_{2A}$ site, labelled by [^{125}I]*R*-(−)DOI (Fig. 2a); however, bufotenine was approximately an order of magnitude more potent at the 5-HT$_{1A}$ site labelled by [^{3}H]8-OH-DPAT (Fig. 2b). Both compounds were 1–2 orders of magnitude less potent at the 5-HT$_{2B}$ receptors labelled by [^{3}H]ketanserin in bovine cortex and both were approximately equipotent at this site (Fig. 2c).

Surprisingly, more data is available on the influence of ring-substituents and *N*,*N*-dialkyl substituents on hallucinogenic activity in man of the indolealkylamines, than is available on their influence on receptor interactions (Shulgin, 1978, 1982; McKenna and Towers, 1984; Nichols and Glennon, 1984; Repke *et al.*, 1985). In general, ring-hydroxylation at the 4-position of the indole nucleus enhances hallucinogenic activity, while hydroxylation at other ring sites reduces or abolishes activity; e.g. psilocin, the 4-hydroxy analog of DMT, is orally active in man at doses of approximately 8–12 mg, while bufotenine, the 5-hydroxy isomer of psilocin, is inactive, probably due to a failure to cross the blood–brain barrier (Nichols and Glennon, 1984; McKenna and Towers, 1984). However, 5-MeO-DMT, the 5-O-methyl analog of bufotenine, is parenterally active as a hallucinogen in man at a dose of approximately 6 mg (Nichols and Glennon, 1984). Repke *et al.* (1985) reported on the activity in man of a series of ring-substituted *N*-methyl,*N*-isopropyl tryptamines. In general, 4-hydroxy-, 4-methoxy- and 5-methoxy-*N*-methyl,*N*-isopropyl tryptamines were substantially more potent than the corresponding *N*,*N*-dimethyl derivatives, while 6-methoxy, 7-methoxy, 5,6-dimethoxy- or 5,6-methylenedioxy-derivatives displayed greatly attenuated activity. 5-Methoxy-*N*-methyl,*N*-isopropyl-tryptamine displayed greatly enhanced stimulant (amphetamine-like) properties in the CNS while visual phenomena (color enhancement, object distortions) were attenuated, compared to the 4-hydroxy congener. Etherification of the 4-position, to give the 4-methoxy congener, also resulted in marked attenuation of the visual component of the effect. In human trials, the $S(+)$ enantiomer of 5-methoxy-alpha-methyltryptamine has been reported as more hallucinogenically active that the *R*-(−) enantiomer (Nichols, 1986). The $S(+)$ enantiomer of alpha-methyltryptamine is more active in animals (Glennon, Young and Jacyno, 1983), but

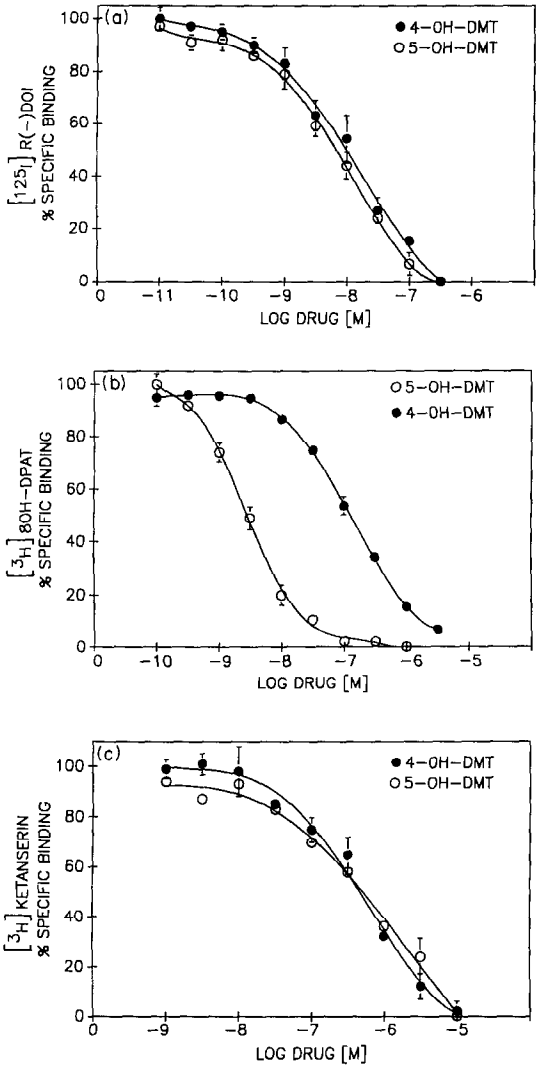


Fig. 2. (a) Radioligand competition curve for 4-hydroxy-*N*,*N*-dimethyltryptamine (psilocin) and 5-hydroxy-*N*,*N*-dimethyltryptamine (bufotenine) at 5-HT$_{2A}$ recognition sites labelled with [^{125}I]*R*-(−)DOI. (b) Radioligand competition curve for 4-hydroxy-*N*,*N*-dimethyltryptamine (psilocin) and 5-hydroxy-*N*,*N*-dimethyltryptamine (bufotenine) at 5-HT$_{1A}$ recognition sites labelled with [^{3}H]8-OH-DPAT. (c) Radioligand competition curve for 4-hydroxy-*N*,*N*-dimethyltryptamine (psilocin) and 5-hydroxy-*N*,*N*-dimethyltryptamine (bufotenine) at 5-HT$_{2B}$ recognition sites labelled with [^{3}H]ketanserin in bovine cortex. Points plotted are means ± standard errors, of 3 independent determinations, performed in triplicate. Standard errors of less than 4% are not shown. Lines plotted are fourth order regressions.

the enantiomers of the unsubstituted derivative have not been evaluated in humans.

It has been shown previously (McKenna and Peroutka, 1989) that phenylisopropylamine hallucinogens have high affinity for the 5-HT_{2A} recognition site, a putative subtype of the 5-HT_2 receptor, and the findings presented here provide evidence to implicate this site in mediating the action of hallucinogenic tryptamines as well. Those derivatives which have been reported as hallucinogenic in human studies also, in general, display enhanced affinities for the 5-HT_{2A} recognition site in the competition studies reported here. A more complete understanding of the structure/activity parameters, determining affinities of putatively hallucinogenic indolealkylamines for 5-HT receptors will require the screening of a larger number of compounds, having systematic variations in substituents at the 4- and 5-position of the indole nucleus.

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