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Comparisons of hallucinogenic phenylisopropylamine binding affinities at cloned human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors

Abstract Since the classical hallucinogens were initially reported to produce their behavioral effects via a 5-HT$_2$ agonist mechanism (i.e., the 5-HT$_2$ hypothesis of hallucinogen action), 5-HT$_2$ receptors have been demonstrated to represent a family of receptors that consists of three distinct subpopulations: 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors. Today, there is greater support for 5-HT$_{2A}$ than for 5-HT$_{2C}$ receptor involvement in the behavioral effects evoked by these agents. However, with the recent discovery of 5-HT$_{2B}$ receptors, a new question arises: do classical hallucinogens bind at 5-HT$_{2B}$ receptors? In the present study we examined and compared the binding of 17 phenylisopropylamines at human 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors. Although there was a notable positive correlation ($r>0.9$) between the affinities of the agents at all three populations of 5-HT$_2$ receptors, structural modification resulted only in small differences in 5-HT$_{2B}$ receptor affinity such that the range of affinities was only about 50-fold. As with 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor affinity, there is a significant correlation ($r>0.9$, $n=8$) between 5-HT$_{2B}$ receptor affinity and human hallucinogenic potency. Nevertheless, given that 5-HT$_{2A}$ and 5-HT$_{2A/2C}$ antagonists – antagonists with low affinity for 5-HT$_{2B}$ receptors – have been previously shown to block the stimulus effects of phenylisopropylamine hallucinogens, it is likely that 5-HT$_{2A}$ receptors play a more prominent role than 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors in mediating such effects despite the affinity of these agents for all three 5-HT$_2$ receptor subpopulations.

Key words Hallucinogenic agents · Serotonin receptors · 5-HT$_2$ receptor subpopulations

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Introduction

Defining the mechanism(s) of action of hallucinogenic agents has been a subject of great interest over the years. Subsequent to the classification of multiple serotonin (5-HT, 5-hydroxytryptamine) receptor subtypes (see Hoyer et al. 1994 for a discussion of 5-HT receptor nomenclature), a significant correlation was found between the affinities of various agents for rat brain 5-HT$_2$ (now 5-HT$_{2A}$) receptors and their reported human hallucinogenic potencies (reviewed: Glennon 1996). This eventually led to the hypothesis that hallucinogens from the ergoline, indolealkylamine, and phenylalkylamine classes, commonly referred to as classical hallucinogens, produce their hallucinogenic effect in humans by activation of 5-HT$_{2A}$ receptors. This hypothesis has been challenged by the finding that these agents also bind at a more recently discovered population of 5-HT receptors (i.e., 5-HT$_{2C}$, formerly 5-HT$_{1C}$ receptors) and that a significant correlation also exists between 5-HT$_{2C}$ receptor affinity and human hallucinogenic potency (Glennon 1996). Although the ergoline and indolealkylamine hallucinogens are relatively nonselective agents that bind at multiple populations of 5-HT receptors, the phenylisopropylamine hallucinogens such as the 1-(2,5-dimethoxy-4X-phenyl)-2-aminopropanes where X = -CH$_3$, -Br, -I (i.e., DOM, DOB, and DOI, respectively), have not been shown to bind with significant affinity at any population of 5-HT receptors except for 5-HT$_2$ receptors (Glennon 1996). Consequently, mechanistic studies have typically focussed on the phenylisopropylamine derivatives.

Classical hallucinogens are agents that (a) produce hallucinogenic effects in humans, (b) substitute for DOM in DOM-trained animals in tests of stimulus generalization, and that (c) bind at 5-HT$_2$ receptors (Glennon 1996). The original 5-HT$_2$ hypothesis related activity to what are now known as 5-HT$_{2A}$ receptors. However, with the identification of the 5-HT$_{2C}$ subpopulation of 5-HT$_2$ receptors, and the subsequently identified correlation between 5-HT$_{2C}$ receptor affinity and hallucinogenic potency (*vide supra*), it became important to determine which (or whether both) of

the two subpopulations of 5-HT$_2$ receptors plays a greater role in the behavioral effects produced by the phenylisopropylamines. We demonstrated that the 5-HT$_2$ antagonist AMI-193, an agent which displays >2,000-fold selectivity for 5-HT$_{2A}$ versus 5-HT$_{2C}$ receptors, potently blocked the stimulus effects of DOM in DOM-trained animals, suggesting that the stimulus effects of DOM are 5-HT$_{2A}$ mediated (Ismaiel et al. 1993). In a similar type of study, Schreiber et al. (1994) were unable to block a DOI stimulus with a 5-HT$_{2C}$-selective antagonist whereas they were successful with a 5-HT$_{2A}$-selective antagonist. Using an antagonist correlation analysis, Fiorella et al. (1995) have implicated a 5-HT$_{2A}$ rather than a 5-HT$_{2C}$ mechanism as mediating the behavioral effects of DOM. Furthermore, *meta*-chlorophenylpiperazine (*m*CPP), a metabolite of the antidepressant trazodone (Ishida et al. 1995), has been examined in humans (e.g., Germine et al. 1994) and there are no reports that this agent produces hallucinogenic effects. *m*CPP is a 5-HT$_{2C}$ agonist (Nelson, unpublished findings) but a 5-HT$_{2A}$ antagonist (Cohen and Fuller 1983). Moreover, *m*CPP has been used as a training drug in animals and its stimulus effects are thought to be 5-HT$_{2C}$ mediated (Callahan and Cunningham 1994). Thus, there is mounting evidence favoring a greater role for 5-HT$_{2A}$ versus 5-HT$_{2C}$ receptors in the stimulus actions of hallucinogenic phenylisopropylamines in rats.

The discovery of a third member of the 5-HT$_2$ family of receptors, 5-HT$_{2B}$ receptors (formerly 5-HT$_{2F}$ receptors; Foguet et al. 1992; Kursa et al. 1992; Loric et at 1992; Wainscott et al. 1993), has now raised the possibility of yet another receptor population as being potentially involved in the actions of these compounds. The cloning of the human form of 5-HT$_{2B}$ receptors (Choi et al. 1994; Kursar et al. 1994; Schmuck et al. 1994; Wainscott et al. 1996) provides an opportunity to examine the binding of phenylisopropylamines at the human version of all three members of the 5-HT$_2$ family to determine the relative pharmacologic specificity across these receptors. Although extensive direct comparisons have not been made, it is clear from existing data that there is considerable overlap among the receptors of the 5-HT$_2$ family (reviewed: Nelson 1993; Westkaemper 1993). The phenylisopropylamines are a well-defined class of hallucinogens for which extensive structure-activity relationships have been established (Glennon 1996). In the present study, a series of representative phenylisopropylamines was evaluated for affinity at the agonist-radiolabeled states of the three human 5-HT$_2$ receptor subtypes to determine whether any pharmacologic specificity exists for these simple molecules across these highly related receptors. Also, for purpose of comparison, 5-HT$_{2B}$ receptor affinities were determined for cloned rat brain receptors to identify if possible species differences exist in the binding of these agents.

Materials and methods

5-HT$_2$ family receptor binding. Radioligand binding to the cloned human 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors was carried out as previously described by Wainscott et al. (1996), and to cloned rat 5-HT$_{2B}$ receptors as described by Kursar et al. (1992). For these assays [^{3}H]5-HT was used to label 5-HT$_{2B}$ receptors and [^{125}I]DOI was used to label 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. Membranes for the radioligand binding assays were prepared from suspension-grown AV12 cells (Syrian hamster fibroblast, ATCC no. CRL 9595) stably transformed with human 5-HT$_{2A}$, 5-HT$_{2B}$, or 5-HT$_{2C}$ receptors as previously described (Wainscott et al. 1996). For the 5-HT$_{2B}$ receptor assay the final [^{3}H]5-HT concentration was approximately 2 nM (range =1.7–2.5 nM), and for the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor assays the final concentration of [^{125}I]DOI was approximately 0.07–0.15 nM. Nonspecific binding was determined in the presence of 1 μM ketanserin for the 5-HT$_{2A}$ assay, 10 μM mianserin for the 5-HT$_{2C}$ receptor assay, and either 10 μM 5-HT or 10 μM 1-naphthylpiperazine (1-NP) for the 5-HT$_{2B}$ assay. Analysis of the competition curves by nonlinear regression analysis to obtain IC$_{50}$ values was as previously described (Wainscott et al. 1996). IC$_{50}$ values from the competition studies were converted to K_i values using the Cheng-Prusoff equation (Cheng and Prusoff 1973). For these calculations the K_d values used for [^{125}I]DOI labeling of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors were 0.65 and 2.4 nM, respectively, and for [^{3}H]5-HT labeling of the 5-HT$_{2B}$ receptor a K_d value of 7.8 nM was used. The K_d values for the radioligands were determined using the receptor binding assays described previously by Wainscott et al. (1996). For the human 5-HT$_{2B}$ receptor the K_d value for [^{3}H]5-HT was determined by saturation analysis and is the mean from eleven separate curves. The K_d values for [^{125}I]DOI binding were determined by the homologous competition method described by Swillens (1992) and represent the mean of nine separate curves at the 5-HT$_{2A}$ receptor and three separate curves for the 5-HT$_{2C}$ receptor.
Drugs. All of the phenylisopropylamines used in the present investigation were synthesized in our laboratories and their synthesis has been reported (Seggel et al. 1990). Agents examined included:

1-(2,5-dimethoxyphenyl)-2-aminopropane HCl (DMA)

1-(2,5-dimethoxy-4-fluorophenyl)-2-aminopropane HCl (DOF)

1-(4-chloro-2,5-dimethoxyphenyl)-2-aminopropane HCl (DOC)

1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane HCl (DOB)

1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI)

1-(2,4,5-trimethoxyphenyl)-2-aminopropane HCl (TMA)

1-(2,5-dimethoxy-4-ethoxyphenyl)-2-aminopropane HCl (MEM)

1-(4-acetyl-2,5-dimethoxyphenyl)-2-aminopropane HCl (DOAc)

1-(2,5-dimethoxy-4-nitrophenyl)-2-aminopropane HCl (DON)

1-(4-cyano-2,5-dimethoxyphenyl)-2-aminopropane HCl (DOCN)

1-(2,5-dimethoxy-4-*n*-propylphenyl)-2-aminopropane HCl (DOPR)

1-(2,5-dimethoxy-4-*n*-hexylphenyl)-2-aminopropane HCl (DOHx)

1-(2,5-dimethoxy-4-*t*-butylphenyl)-2-aminopropane HCl (DOTB)

1-(4-benzyl-2,5-dimethoxyphenyl)-2-aminopropane HCl (DOBz)

N,N-dimethyl-1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane HBr (M-154)

N-n-propyl-1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane HCl (D-367)

N,N,N-trimethyl-1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane iodide (QDOB)

All compounds were homogeneous by thin-layer chromatography and melting points were identical to those reported earlier. *Meta*-chlorophenylpiperazine (*m*CPP) was used as the HCl salt.

[^{3}H]5-HT (22.8–26.7 Ci/mmol) and [^{125}I]DOI (2,200 Ci/mmol) were purchased from Dupont-NEN (Wilmington, Del., USA). All other chemicals for the radioligand binding assays were obtained from sources previously described (Wainscott et al. 1996).
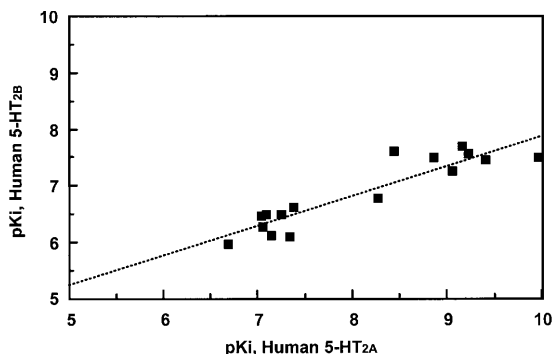
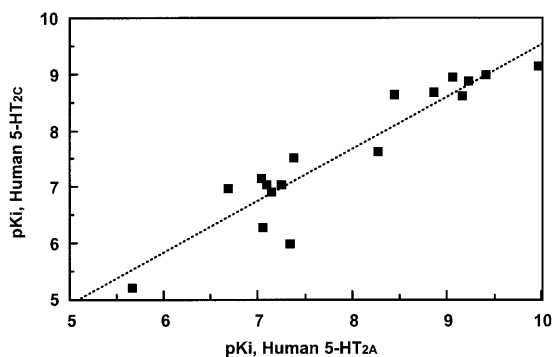**Fig. 1** A comparison of human 5-HT$_{2A}$ and human 5-HT$_{2B}$ binding data (slope =0.533, r=0.920, n=16)

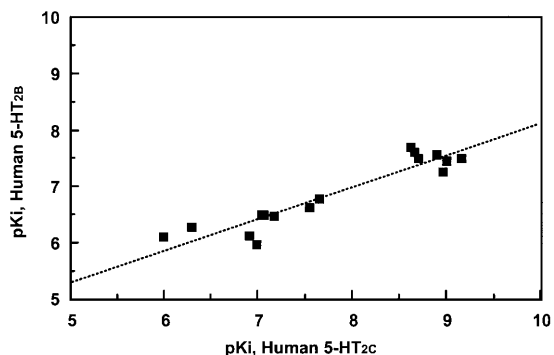**Fig. 2** A comparison of human 5-HT$_{2A}$ and human 5-HT$_{2C}$ binding data (slope =0.937, r=0.935, n=17)

Fig. 3 A comparison of human 5-HT$_{2C}$ and human 5-HT$_{2B}$ binding data (slope =0.554, r=0.938, n=16)
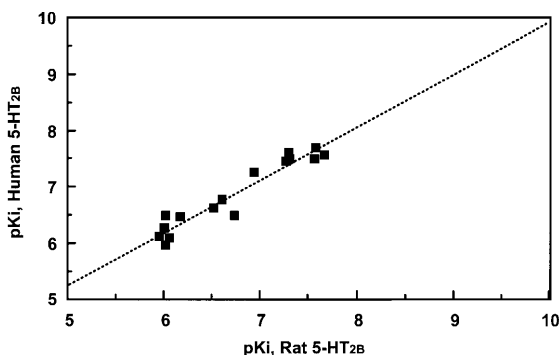

Fig. 4 A comparison of rat 5-HT$_{2B}$ and human 5-HT$_{2B}$ binding data (slope =0.925, r=0.960, n=16)

Results

Comparisons of human 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ binding

Table 1 shows the affinities of 17 phenylisopropylamines at human 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors. Comparisons of affinities among all three of the human 5-HT$_2$ receptors (Figs. 1–3) showed strong positive correlations. In the case of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, the correlation of the affinities gave a slope near unity (Fig. 2) indicating a good one-to-one relationship for absolute affinities. This is borne out by examination of the ratios of 5-HT$_{2C}$ to 5-HT$_{2A}$ K_i values in Table 1 where most of the ratios are clustered around unity. The single exception was DOCN which had a 22-fold higher affinity for 5-HT$_{2A}$ versus 5-HT$_{2C}$ receptors.

In contrast to the comparison between 5-HT$_{2A}$ and 5-HT$_{2C}$ binding affinities, the slopes of the correlation plots for 5-HT$_{2B}$ receptor binding against either 5-HT$_{2A}$ (Fig. 1) or 5-HT$_{2C}$ (Fig. 3) binding showed slopes closer to 0.5 than to unity. Thus, although there was a good correlation based on rank order of affinity, there were significant differences in absolute affinities for a number of the compounds. Examination of the compounds and their affinities showed that the shallow slopes were because structural changes that dramatically increased 5-HT$_{2A}$ and 5-HT$_{2C}$ affinities had a much more modest effect on 5-HT$_{2B}$ affinity. The highest affinity compounds at 5-HT$_{2B}$ receptors displayed K_i values in the approximate range of 25–35 nM whereas these same compounds displayed subnanomolar affinities at 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors.

Affinities are also shown (Table 1) for rat 5-HT$_{2B}$ (r5-HT$_{2B}$) receptors to determine the possibility of any species differences of phenylisopropylamines at these receptors. Previous work (Johnson et al. 1994; Wainscott et al. 1996) had identified groups of antagonist structures that exhibited differences in affinity between rat and human forms of 5-HT$_{2B}$ receptors. However, the phenylisopropylamines
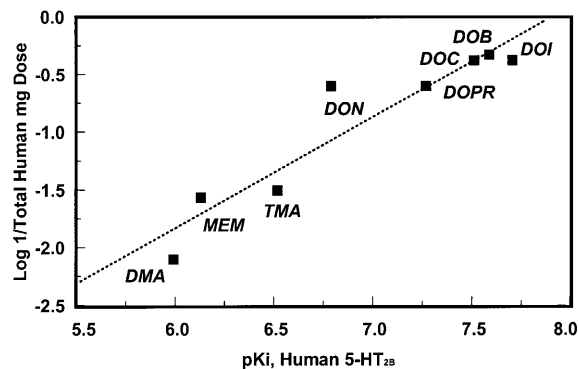

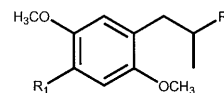**Fig. 5** Relationship between 5-HT$_{2B}$ receptor affinity (pK_i) and human hallucinogenic potency for eight phenylisopropylamines (slope =0.958, r=0.941). Human data, as total human mg dose, are from Jacob and Shulgin (1994) for DOB (1–3 mg), DOI (1.5–3 mg), DOC (1.5–3 mg), DOPR (2.5–5 mg), DON (3–4.5 mg), TMA (20–40 mg), MEM (20–50 mg), and DMA (80–160 mg); the arithmetic mean was employed where dose ranges were provided

Table 1 Affinities of phenylisopropylamines at agonist-labeled members of the 5-HT$_2$ receptor family. The 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors were labeled with [^{125}I]DOI and the 5-HT$_{2B}$ receptors with [^{3}H]5-HT. Each value is the mean of three separate experiments

Substituents			K_i values (± SEM)(nM)			Binding ratios			K_i (± SEM)(nM)	Ratio
R1	R2	Name	Human 5-HT$_{2A}$	Human 5-HT$_{2B}$	Human 5-HT$_{2C}$	Ratio 2C/2A	Ratio 2B/2A	Ratio 2B/2C	Rat 5-HT$_{2B}$	h5-HT$_{2B}$/ r5-HT$_{2B}$
-H	-NH$_2$	DMA	211 (15)	1039 (143)	104 (12)	0.5	4.9	10.0	950 (24)	1.1
-F	-NH$_2$	DOF	41.7 (1.8)	227 (15)	28.7 (1.5)	0.7	5.4	7.9	306 (26)	0.7
-Cl	-NH$_2$	DOC	1.4 (0.04)	31.8 (2.2)	2.0 (0.2)	1.5	22.7	15.9	26.8 (1.5)	1.2
-Br	-NH$_2$	DOB	0.6 (0.1)	26.9 (4.6)	1.3 (0.2)	2.2	44.8	20.1	21.8 (1.5)	1.2
-I	-NH$_2$	DOI	0.7 (0.06)	20.0 (3.6)	2.4 (0.3)	3.6	28.6	8.5	26.6 (1.6)	0.8
-OCH$_3$	-NH$_2$	TMA	57.9 (3.3)	307 (21)	87.7 (2.0)	1.5	5.3	3.5	187 (16)	1.6
-OC$_2$H$_5$	-NH$_2$	MEM	73.0 (10.0)	763 (54)	124 (8)	1.7	10.5	5.2	1130 (237)	0.7
-COCH$_3$	-NH$_2$	DOAc	80.5 (12.1)	313 (27)	91.3 (8.4)	1.1	3.9	3.4	947 (5)	0.3
-NO$_2$	-NH$_2$	DON	5.5 (0.6)	166 (28)	22.4 (1.5)	4.1	30.2	7.4	248 (60)	0.7
-CN	-NH$_2$	DOCN	45.7 (0.9)	774 (46)	1011 (7)	22.1	16.9	0.8	877 (169)	0.9
-nC$_3$H$_7$	-NH$_2$	DOPR	0.9 (0.01)	54.4 (10.3)	1.1 (0.1)	1.2	60.4	49.5	115 (16)	0.5
-nC$_6$H$_{13}$	-NH$_2$	DOHx	0.1 (0.03)	30.3 (12.5)	0.7 (0.05)	7.0	303	43.3	49.0 (12.7)	0.6
-tC$_4$H$_9$	-NH$_2$	DOTB	3.7 (0.2)	24.6 (6.5)	2.2 (0.3)	0.6	6.7	11.2	49.6 (7.1)	0.5
-CH$_2\phi$	-NH$_2$	DOBz	0.4 (0.08)	35.0 (3.3)	1.0 (0.1)	2.9	87.5	35.0	53.8 (6.8)	0.7
-Br	-N(CH$_3$)$_2$	M-154	94.2 (6.8)	341 (49)	68.1 (2.)	0.7	3.6	5.0	683 (59)	0.5
-Br	-NH-C$_3$H$_7$	D-367	88.5 (5.8)	521 (21)	514 (6)	5.8	5.9	1.0	976 (25)	0.5
-Br	-N$^+$(CH$_3$)$_3$	QDOB	2155 (56)	>10,000	6298 (165)	2.9	–	–	>10,000	–

showed no indication of species differences in their binding at the two 5-HT$_{2B}$ receptors (Fig. 4).

*m*CPP was found to bind at each of the three populations of human 5-HT$_2$ receptors; 5-HT$_{2A}$ K_i=32.1 (±3.7) nM, 5-HT$_{2B}$ K_i=28.8 (±1.9) nM, and 5-HT$_{2C}$ K_i=3.4 (±0.3) nM.

Discussion

The members of the 5-HT$_2$ family of receptors share significant sequence homology, especially within the transmembrane portions of the receptor which are thought to be important for ligand recognition. The present comparison of the human forms of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors illustrates the relatedness of the agonist-labeled recognition domains of these receptor subpopulations and lends support to structural similarities predicted on the basis of molecular graphics models (Westkaemper 1993). Also consistent with previous findings on the binding of related agents at rat brain 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors (Glennon et al. 1992), a comparison of K_i values (Table 1) revealed no significant discrimination among the group of compounds examined herein at human 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors.

The present investigation also demonstrates that phenylisopropylamines can bind with high affinity at 5-HT$_{2B}$ receptors. Examination of 5-HT$_{2B}$ binding revealed significant correlations with 5-HT$_{2A}$ and 5-HT$_{2C}$ binding data; however, this was not a one-to-one relationship. Whereas a number of the phenylisopropylamines displayed sub-

nanomolar afinities for 5-HT$_{2A}$ receptors, and single-digit nanomolar affinities for 5-HT$_{2C}$ receptors, the highest affinity phenylisopropylamine for 5-HT$_{2B}$ receptors (i.e., DOI) displayed an affinity of only 20 nM. These findings suggest that there are important differences in the composition of the ligand binding domains of 5-HT$_{2B}$ versus 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. Furthermore, in contrast to what was seen at 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, variation of the 4-position substituent had relatively less impact on 5-HT$_{2B}$ binding affinity. Phenylisopropylamines with hydrophobic 4-position substituents (e.g., DOPR, DOHx, DOBz) displayed some selectivity for 5-HT$_{2A/2C}$ versus 5-HT$_{2B}$ receptors and may provide leads for the development of agents with greater selectivity. One caveat to the present studies is that the results are based solely on radioligand binding data which provide no insight into the agonist, partial agonist, or antagonist nature of these compounds at 5-HT$_{2B}$ receptors. Additional studies using functional assays will be required to determine the influence of 4-position substituents on efficacy.

Effects of structural changes on 5-HT$_2$ receptor affinity

The 2,5-dimethoxy substitution pattern of the phenylisopropylamines has been already established as contributing to high affinity for rat brain 5-HT$_2$ receptors, and modification of the 4-position substituent has been demonstrated to further modulate affinity over a wide range (Glennon 1996). Consequently, we focussed primarily on the effect of 4-position modification while keeping the 2,5-

dimethoxy substituents constant. Terminal amine substitution has also been previously shown to result in reduced affinity for 5-HT$_2$ receptors; thus, this too was briefly explored in the present study. The most obvious difference among the binding results upon cursory inspection of the data in Table 1 is that whereas the K_i values span a 1,700-fold range (excluding QDOB) at 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, the range of K_i values for 5-HT$_{2B}$ binding is only about 50-fold. Furthermore, although the higher affinity compounds bind at 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors with sub-nanomolar affinities, the higher affinity agents for 5-HT$_{2B}$ receptors bind with K_i values only in the 25–35 nM range. It would seem, then, that as a class these agents bind with lower affinities at 5-HT$_{2B}$ receptors than at 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors and that 5-HT$_{2B}$ affinity is relatively less influenced by structural modifications of the type investigated in the present study.

Structure-activity relationships that can be formulated for 5-HT$_{2A}$ binding from the present results are essentially identical to those that have been previously published for the binding of these agents at rat and human 5-HT$_{2A}$ receptors for a related and partially overlapping series of agents (Glennon 1996; Glennon et al. 1992). Likewise, the significantly parallel binding affinities of phenylisopropylamines at rat 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors has afforded nearly identical structure-activity conclusions (Glennon et al. 1992) that are consistent with the results of the present study. In general, DMA (which bears a 4-H group) is the lowest affinity agent of the series; replacement of hydrogen with substituents of greater hydrophobic and/or electron withdrawing nature results in enhanced affinity.

Formulation of structure-activity relationships for 5-HT$_{2B}$ binding is made difficult by the fairly refractive nature of the influence of substituent groups on affinity. Nevertheless, DMA still represents the lowest affinity primary amine in the series. Introduction of substituents tends to result in enhanced affinity especially when the substituents are of a hydrophobic or electron withdrawing nature, but more polar substituents (e.g., as with TMA, MEM, DOAc, DON, and DOCN) result in lower affinity agents.

As has been previously demonstrated for 5-HT$_{2A}$ and 5-HT$_{2C}$ binding, primary amines are optimal for 5-HT$_{2B}$ binding. A comparison of a primary amine (DOB) with a related secondary amine (D-367), a tertiary amine (M-154), and a quaternary amine (QDOB) reveals that each of the latter agents binds with reduced affinity.

5-HT$_2$ receptors and behavioral activity

By themselves, the human binding data shown in Table 1 provide little or no insight into which of the 5-HT$_2$ subpopulations is most important for the hallucinogenic or behavioral activity of the phenylisopropylamines. If anything, the data implicate a potential role for yet another population of receptors: 5-HT$_{2B}$ receptors. Indeed, for eight phenylisopropylamines for which human data were available (Jacob and Shulgin 1994), 5-HT$_{2B}$ receptor affinity is highly correlated (r=0.941) with human hallucinogenic potency (Fig. 5). This was not altogether unexpected given the relationship between 5-HT$_{2A}$ and 5-HT$_{2B}$ binding (Fig. 1). Furthermore, curiously enough, over 15 years ago it was reported that a significant relationship exists between the hallucinogenic potencies of various indolealkylamines and phenylalkylamines and their affinities for the serotonin receptors of the rat fundus preparation (reviewed: Glennon 1985). These rat fundus 5-HT receptors are now thought to be primarily of the 5-HT$_{2B}$ type (e.g., Foguet et al. 1992; Kursar et al. 1992). Together with previous results (Glennon 1996), it now appears that human hallucinogenic potency correlates with 5-HT$_2$ receptor affinity regardless of which of the three 5-HT$_2$ subpopulations is considered.

Nevertheless, there is still some evidence that favors 5-HT$_{2A}$ receptors as being significant contributors to the behavioral effects of the phenylisopropylamines. In humans, there is no indication that mCPP produces hallucinogenic effects (Germine et al. 1994). mCPP binds at all three populations of human 5-HT$_2$ receptors; it displays 10-fold selectivity for 5-HT$_{2C}$ versus 5-HT$_{2A}$ receptors, but behaves as a 5-HT$_{2C}$ agonist and a 5-HT$_{2A}$ antagonist (Nelson, unpublished findings). Taken together with the animal studies described in the Introduction, these findings are inconsistent with 5-HT$_{2C}$ receptors being primarily or solely responsible for the hallucinogenic effects of the phenylisopropylamines. What about a role for 5-HT$_{2B}$ receptors? AMI-193, the 5-HT$_2$ antagonist that blocked the stimulus effects of DOM in DOM-trained animals and which displays 2,000-fold selectivity for 5-HT$_{2A}$ versus 5-HT$_{2C}$ receptors, has been recently demonstrated to bind with 100-fold selectivity for 5-HT$_{2A}$ versus 5-HT$_{2B}$ receptors (Ismaiel et al. 1996). Furthermore, the standard 5-HT$_{2A/2C}$ antagonists ketanserin and pirenperone, which potently blocked the behavioral effects of the hallucinogenic phenylisopropylamines (Glennon 1992, 1996), bind with >1000-fold selectivity at 5-HT$_{2A}$ over 5-HT$_{2B}$ receptors (Nelson 1993). These findings would suggest that the behavioral effects, and particularly the DOM-stimulus effects, produced by hallucinogenic phenylisopropylamines are not 5-HT$_{2B}$ mediated, which might have been expected given the localization of 5-HT$_{2B}$ receptors in brain.

It is impossible to draw any conclusions about human hallucinogenic activity until the appropriate human experiments have been conducted. Nevertheless, given the significant correlation that exists between the stimulus generalization potencies of the phenylisopropylamines in DOM-trained rats and their hallucinogenic potencies in humans, as well as the similarity between the binding of the phenylisopropylamines at (a) human 5-HT$_{2A}$ and human 5-HT$_{2B}$ receptors, and between (b) rat and human 5-HT$_{2B}$ receptors, it would appear that 5-HT$_{2A}$ receptors are the most likely candidate member of the 5-HT$_2$ family for mediation of hallucinogenic effects.

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