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Radioligand binding evidence implicates the brain 5-HT_2 receptor as a site of action for LSD and phenylisopropylamine hallucinogens

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Abstract. Alterations in brain serotonergic function have been implicated in the mechanism of action of LSD, mescaline, and other similarly acting hallucinogenic drugs of abuse such as STP (2,5-dimethoxyphenylisopropylamine; DOM). In order to test the hypothesis that the mechanism of action of LSD and phenylisopropylamine hallucinogens is through stimulation of a specific brain serotonin receptor sub-type, the affinities of these compounds for radiolabelled 5-HT_2, 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} receptors have been determined using recently developed in vitro radioligand binding methodologies. The 5-HT_2 receptor was labelled with the agonist/hallucinogen radioligand ^{3}H-DOB (4-bromo-2,5-dimethoxyphenylisopropylamine). The 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} receptors were labelled with ^{3}H-OH-DPAT, ^{3}H-5-HT, and ^{3}H-mesulergine, respectively. In general, the phenylisopropylamines displayed 10–100 fold higher affinities for the 5-HT_2 receptor than for the 5-HT_{1C} receptor and 100–1000 fold higher affinities for the 5-HT_2 receptor than for the 5-HT_{1A} or 5-HT_{1B} receptor. There was a strong correlation between hallucinogenic potencies and 5-HT_2 receptor affinities of the phenylisopropylamines ($r=0.90$); the correlation coefficients for the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} were 0.73, 0.85, and 0.78, respectively. Because there is no evidence that 5-HT_{1A}-selective or 5-HT_{1B}-selective agonists are hallucinogenic and because the phenylisopropylamines are potent hallucinogens, a 5-HT_2 receptor interaction is implicated and supports our previous suggestions to this effect. A secondary role for 5-HT_{1C} receptors cannot be discounted at this time. These results, when integrated with other receptor pharmacological information, indicate that an important component of the mechanism of action of LSD and the phenylisopropylamine hallucinogens is through stimulation of brain 5-HT_2 receptors.

Key words: 5-HT_2 receptor – LSD – Phenylisopropylamines – Hallucinogens

The mechanism of action underlying the hallucinogenic and/or psychoactive properties of LSD has been an intriguing mystery for more than 40 years. Evidence pointing to the involvement of serotonergic systems was provided by the electrophysiological studies of Aghajanian et al. (1968, 1970, 1972). However, the identification of a pharmacologically defined receptor as the specific site of action of LSD accounting for its potent mind-altering effects has been elusive.

The phenylisopropylamine hallucinogens produce a syndrome that is apparently very similar to the spectrum of effects produced by LSD (Shulgin 1978). One strategy for uncovering a specific hallucinogenic site of action for LSD has been to identify a common site of action of the phenylisopropylamine hallucinogens and LSD. Extensive structure-activity studies (SAR) have been pursued involving testing the hallucinogenic and/or psychoactive potencies of these compounds (Nichols and Glennon 1984). Also SAR studies involving testing the potencies of the phenylisopropylamines as behavioral cues in rats trained to bar-press when administered the hallucinogenic phenylisopropylamine DOM (2,5-dimethoxyphenylisopropylamine) have been pursued (for review, see Glennon et al. 1984a). A strong correlation has been demonstrated between the hallucinogenic potencies of phenylisopropylamines and their potencies in cueing DOM-trained rats (Glennon et al. 1984b), indicating that the DOM drug-discrimination model involves a brain drug receptor that may be the key site in the production of the psychoactive effects in humans.

Recently a highly significant correlation was demonstrated between the potencies of the phenylisopropylamines as either hallucinogens or behavioral cues and their affinities for ^{3}H-antagonist radiolabelled 5-HT_2 receptors (Glennon et al. 1984b). However, there are at least three additional distinct serotonin receptor binding sites in rat brain detectable with radioligand binding assays. For this reason, it was important to investigate the relationships between the biological potencies of the phenylisopropylamines and their affinities for the radiolabelled 5-HT receptor subtypes. Furthermore, a radioactive hallucinogen, ^{3}H-DOB, that apparently labels the 5-HT_2 receptor with high affinity and specificity has recently been developed (Titeler et al. 1985; Lyon et al. 1987). Agonist interactions with 5-HT_2 receptors are 10–100 fold more potent using this radioligand than the antagonist radioligands previously used to label the 5-HT_2 receptor. Serotonin displays nanomolar affinity for the 5-HT_2 receptor when ^{3}H-DOB is used as the radiolabel (Titeler et al. 1985; Lyon et al. 1987). It is presumed that the 5-HT_1 receptor radioligands are also labelling an agonist high affinity state, as serotonin displays a nanomolar affinity for these receptors. Thus, it was of interest to determine if phenylisopropylamine hallucinogens

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would display higher affinities using ^{3}H-DOB as the 5-HT$_2$ radioligand rather than ^{3}H-ketanserin.

Materials and methods

Tissue preparation and radioligand binding assays procedures were as described previously with minor modifications (Battaglia et al. 1984; Lyon et al. 1987). Following decapitation the brains of male Sprague-Dawley rats were removed and placed in 0.9% ice-cold saline and then dissected over ice until the tissue was prepared. Tissues were stored in ice-cold saline for no longer than 1 h and following blot drying and weighing they were prepared and frozen at −30° C until used. Freshly dissected tissue was homogenized (Polytron setting 6 for 20 s) in 30 vol ice-cold buffer containing 50 mM Tris-HCl (pH 7.4 at 37° C), 0.5 mM Na_2EDTA an 10 mM $MgSO_4$ and centrifuged at 30000 *g* for 15 min. The supernatant was discarded and the pellet resuspended and pre-incubated for 15 min at 37° C. The homogenate membranes were washed twice by centrifugation and resuspension. The final suspension buffer contained 10 μM pargyline and 0.1% ascorbate and was added last to the assay incubation medium. The agonist high affinity state of the 5-HT$_2$ receptor was labelled with 0.4 nM ^{3}H-DOB and 20 mg homogenized rat frontal cortical tissue (Lyon et al. 1987). Cinanserin 10^{-6} M was used to define non-specific binding. The 5-HT$_{1A}$ receptor was labelled with 0.1 nM ^{3}H-8-OH-DPAT and 2.5 mg rat hippocampal homogenates. 8-OH-DPAT 10^{-6} M was used to define non-specific binding. The 5-HT$_{1B}$ receptor was labelled with 2.0 nM ^{3}H-serotonin and 2.5 mg rat striatal membrane homogenates. 5-HT 10^{-6} M was used to define non-specific binding and 10^{-7} M 8-OH-DPAT and 10^{-7} M mesulergine were included to block 5-HT$_{1A}$ and 5-HT$_{1C}$ receptors, respectively. 5-HT$_{1C}$ receptors were labelled with 1.0 nM ^{3}H-mesulergine in the presence of 20 nM spiperone with 20 mg pig cortical membranes. Non-specific binding was determined using 10 μM 5-HT. Eleven concentrations of non-radioactive competing drugs were made fresh daily in assay buffer. Following incubation with membranes and radioligand at 37° C for 15 min (for ^{3}H-DOB assays) or 30 min (for 5-HT$_1$ assays) samples were rapidly filtered over glass fibre filters and washed with 10 ml ice-cold 50 mM Tris-HCl buffer. Individual filters were inserted into vials and equilibrated with 5 ml scintillation fluid for 6 h before counting at 50% efficiency. Results were analyzed using an updated version of the program EBDA (McPherson 1983) and statistically analyzed using RS1 (BBN Software). Serotonin receptor affinities were determined using ^{3}H-DOB (5-HT$_2$), ^{3}H-8-OH-DPAT (5-HT$_{1A}$), ^{3}H-5-HT (5-HT$_{1B}$) and ^{3}H-mesulergine (5-HT$_{1C}$). The values reported are the K_i values determined from experimentally determined Ic_{50} values, adjusted for the presence of radioligand using the Cheng-Prusoff equation (Cheng and Prusoff 1973).

Results and discussion

Table 1 lists the K_I values determined using the four radioligand binding assays utilized in this study (see Materials and methods). Table 1 also lists the approximate human dosage of phenylisopropylamines that produce psychoactive effects and the dosages that cue DOM-trained rats (Nichols and Glennon 1984; Glennon et al. 1984b). LSD displays high affinity for all four serotonin receptor sub-types. However, the potent hallucinogen DOB displays an approximatley 100 fold higher affinity for the 5-HT$_2$ receptor than for the 5-HT$_{1C}$ receptor, 2000-fold higher affinity for the 5-HT$_2$ receptor than for the 5-HT$_{1B}$ and 5000-fold higher affinity for the 5-HT$_2$ receptor than for the 5-HT$_{1A}$ receptor. This pattern is maintained throughout the entire series of phenylisopropylamine hallucinogens (Table 1). These data indicate that although LSD displays high affinity for all the serotonin receptor sub-types, only the 5-HT$_2$ receptor displays high affinity for both the potent phenylisopropylamine hallucinogens and LSD.

The correlation coefficients (*r*) are also reported in Table 1 for receptor affinities versus either psychoactive potency (in humans) or behavioral potency (i.e., stimulus generalization in DOM-trained rats). While the 5-HT$_2$ receptor affinities displayed the highest degree of correlation with either human psychoactive potencies or DOM discrimination potencies, the affinities of the phenylisopropylamines and LSD for the other serotonin receptor sub-types also resulted in significant correlations. However, it should be noted that the correlations involving the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors involved substances that displayed no detectable affinities in vitro (K_I values >10000 nM) for the receptors and yet produced significant effects in humans and in DOM-trained animals (Table 1). In addition, results of clinical trials with 5-HT$_{1A}$-selective (e.g., buspirone, Gammans et al. 1986) and 5-HT$_{1B}$-selective (e.g., mCPP, Mueller et al. 1986) agonists suggest that these agents are not hallucinogenic in humans. Furthermore, in drug discrimination studies using animals trained to discriminate various hallucinogens and serotonin agonists from vehicle, stimulus generalization did not occur between the hallucinogens and 5-HT$_{1A}$ or 5-HT$_{1B}$ agonists regardless of which was used as the training drug, and the effects of hallucinogens (but not those of 5-HT$_{1A}$ or 5-HT$_{1B}$-selective agonists) can be antagonized by 5-HT$_2$ antagonists (Glennon 1987). In addition, as indicated above, the affinities of the phenylisopropylamines for the non-5-HT$_2$ receptors were orders of magnitude lower than for the 5-HT$_2$ receptor. Taken together, these observations indicate that the correlations are due to some degrees of similarity in the binding sites of these receptors, which is not surprising as they are all receptors for the same neurotransmitter, serotonin. Furthermore, the degree of similarity seems to be greatest for the 5-HT$_2$ and 5-HT$_{1C}$ receptors, as they display the highest affinities for the phenylisopropylamines and have the strongest degree of correlation ($r=0.88$ for 5-HT$_2$ versus 5-HT$_{1C}$). There were no statistically significant correlations between K_I values for the other receptors. Supportive of the argument that the 5-HT$_2$ and the 5-HT$_{1C}$ receptor are more closely related to each other than to the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors are the observations that both the 5-HT$_2$ and 5-HT$_{1C}$ receptors apparently mediate stimulation of phosphoinositol metabolism (Sanders-Bush and Conn 1986). 5-HT$_{1A}$ receptors have been reported to inhibit adenylate cyclase activity [14]. It is not yet known what intracellular events are altered by 5-HT$_{1B}$ receptor stimulation.

Significant correlations have been observed between phenylisopropylamine hallucinogenic potencies and 5-HT$_2$ receptor affinities using ^{2}H-ketanserin, an antagonist, non-hallucinogenic radiolabel (Glennon et al. 1984a). However, the apparent affinities of the phenylisopropylamines were

Table 1. Serotonin receptor affinities, human hallucinogenic potencies and drug-discrimination values

Agent		5-HT_2 (n^M)	5-HT_{1A} (n^M)	5-HT_{1B} (n^M)	5-HT_{1C} (n^M)	[a]	[b]
1.	*d*-LSD	0.54± 0.07	0.43 ± 0.02	6.6 ± 0.03	3.8± 0.6	6.70	7.0
2.	R(−) DOB	0.39± 0.11	2332 ±188	683 ± 46	47 ± 10	5.80	6.5
3.	DOI	0.70± 0.07	2355 ± 77	1261 ±105	30 ± 4	5.40	6.0
4.	DOB	0.79± 0.01	3770 ±118	831 ± 37	69 ± 16	5.35	6.19
5.	DOPR	0.90± 0.19	2849 ±170	2330 ±101	14 ± 1	5.26	6.21
6.	R(−) DOM	1.80± 0.40	4004 ±107	1840 ±172	94 ± 17	5.15	6.06
7.	DOET	1.50± 0.30	3930 ±115	2451 ±226	101 ± 20	4.98	6.05
8.	DOM	8 ± 0.40	5122 ±140	2063 ±112	193 ± 20	4.85	5.75
9.	S(+) DOB	2.30± 0.20	4041 ±156	883 ± 49	81 ± 7	4.80	5.58
10.	DOBU	1.70± 0.30	4178 ±165	1211 ± 86	26 ± 5	4.46	5.49
11.	2,4,5 TMA	81 ± 6	>10000	>10000	2666 ± 76	4.12	4.86
12.	MEM	113 ± 8	>10000	>10000	2278 ± 90	3.96	4.64
13.	2,5 DMA	268 ±32	1131 ± 55	8435 ±668	1217 ± 89	3.66	4.62
14.	2,4 DMA	234 ±29	>10000	>10000	3152 ± 83	3.59	4.68
15.	R(−) MDA	198 ±37	4167 ±110	>10000	2079 ± 48	3.49	5.42
16.	MDA	387 ±54	5644 ±114	>10000	2932 ± 88	3.24	5.10
17.	3,4,5 TMA	307 ±32	>10000	>10000	5710 ±150	3.14	4.61
Correlations:							
Human potency (*r*)		0.90	0.73	0.85	0.78		
Drug discrimination (*r*)		0.90	0.69	0.80	0.82		

[a] log values of human hallucinogenic potencies (μmole)
[b] log values of ED_{50} values in animal drug discrimination studies (μmole/kg)

r refers to Spearman's rank correlation coefficient. Structures of these compounds may be found in Shulgin (1978) and Shannon et al. (1984). The receptors listed were radiolabelled as indicated in Materials and methods.

generally 10–100 fold lower using ^{3}H-ketanserin than is observed using ^{3}H-DOB. Previous work has indicated that the 5-HT_2 receptor is a two-state receptor capable of forming a complex with a GTP-binding protein (Battaglia et al. 1984; Titeler et al. 1984). ^{3}H-DOB apparently labels a guanine nucleotide-sensitive agonist high-affinity state of the 5-HT_2 receptor (Lyon et al. 1987). Experience with many receptors that interact with GTP-binding proteins has shown that the relative affinities of agonists at the two states are identical; the absolute affinity is different (Titeler 1987). Therefore there should be a similar degree of correlation between hallucinogenic potency versus ^{3}H-ketanserin binding affinity and hallucinogenic potency and ^{3}H-DOB binding affinity. The advantages of using ^{3}H-DOB in this study derive from the ability to demonstrate the extremely high affinities the phenylisopropylamines display in interacting with the 5-HT_2 receptor and the fact that this radioligand provides a direct demonstration of the high affinity interaction of a radioactive phenylisopropylamine hallucinogen with the 5-HT_2 receptor.

In summary, it has been demonstrated herein that the phenylisopropylamine hallucinogens interact with high affinity and with a high degree of correlation (versus their hallucinogenic and behavioral potencies) only with the 5-HT_2 receptor. A somewhat less significant correlation is obtained with 5-HT_{1A} and 5-HT_{1B} binding; however, other pharmacological data suggest that these sites may not play a primary role in the mechanism of action of hallucinogenic agents. On the other hand, a role for 5-HT_{1C} sites is unclear at this time. As LSD has similar hallucinogenic and behavioral effects as the phenylisopropylamines, and demonstrates high affinity for the 5-HT_2 receptor, it seems likely that the psychoactive effects of the phenylisopropylamine hallucinogens and LSD are mediated through stimulation of brain 5-HT_2 receptors. The extremely potent hallucinogenic effects of LSD may indicate a secondary involvement of a 5-HT_1 receptor sub-type in the mechanism of action of LSD. The radioligand binding evidence also indicates a degree of similarity between the binding interactions of phenylisopropylamines to the 5-HT_2 and 5-HT_{1C} receptors and suggests that these two receptors may be more closely related to each other than to other serotonin receptor subtypes.

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