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Hallucinogenic drug interactions at human brain 5-HT$_2$ receptors: implications for treating LSD-induced hallucinogenesis

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Abstract. It has been shown that the hallucinogenic potencies of LSD, the phenylisopropylamines, such as DOB (4-bromo-2,5-dimethoxyphenylisopropylamine) and DOI (4-iodo-2,5-dimethoxyphenylisopropylamine), and the indolealkylamines, such as DMT (dimethyltryptamine) and 5-OMe-DMT (5-methoxy-dimethyltryptamine), strongly correlate with their in vitro 5-HT$_2$ receptor binding affinities in rat cortical homogenates. In order to ascertain if this correlation applies to human 5-HT$_2$ receptors as well, we examined the affinities of 13 psychoactive compounds at ^{3}H-ketanserin-labelled 5-HT$_2$ receptors in human cortical samples. Both radioligand binding and autoradiographical procedures were used. As in rat brain, *d*-LSD was the most potent displacer of ^{3}H-ketanserin specific binding with a K_i of 0.9 nM. The phenylisopropylamine DOI also displayed high affinity (K_i of 6 nM). Stereospecific interactions were found with DOB; (−) DOB had a K_i of 17 nM while (+) DOB had a K_i of 55 nM. The behaviorally active compound DOM (4-methyl-2,5-phenylisopropylamine) had an affinity of 162 nM while its behaviorally less active congener iso-DOM had an affinity of 6299 nM. The indolealkylamines 5-OMe-DMT and DMT competed with moderate affinities (207 and 462 nM, respectively). In general, Hill coefficients were significantly less than unity which is consistent with an agonist interaction with 5-HT$_2$ receptors. MDMA, a substituted amphetamine analog was inactive with a K_i of greater than 10 μM. A strong correlation was found for the hallucinogen affinities and human hallucinogenic potencies ($r = 0.97$). Also, human and rat brain 5-HT$_2$ receptor affinities were strongly correlated ($r = 0.99$). These results strongly support the hypothesis that the hallucinogenic effects of these drugs in humans are mediated in whole or in part via 5-HT$_2$ receptors. Furthermore, these studies imply that treatment with 5-HT$_2$ receptor antagonists may be effective in reversing the hallucinogenic effects caused by the ingestion of LSD and LSD-like drugs.

Key words: Hallucinogen – DOB – DOI – 5-HT$_2$ receptor – LSD

Over the past few years pharmacological studies have suggested a major role for the central serotonergic system in mediating the effects of the psychoactive (hallucinogenic) compounds such as LSD, the 2,5 substituted phenylisopropylamines (such as DOB and DOI) and the dimethyltryptamines (such as DMT and 5-OMe-DMT). In addition, behavioral studies (Glennon et al. 1983), electrophysiological studies (Rasmussen and Aghajanian 1988) and radioligand binding studies (Shannon et al. 1984; Lyon et al. 1987) indicate that these hallucinogenic drugs are 5-HT$_2$ receptor agonists. In rat drug discrimination studies it was shown that rats trained to discriminate DOM (4-methyl-2,5-dimethoxyphenylisopropylamine) recognized and responded in a dose-dependent manner to such agents as *d*-LSD, DOI and DOB (for review see Glennon et al. 1984a). This stimulus generalization was potently blocked by the 5-HT$_2$ receptor antagonists ketanserin and pirenperone (Glennon et al. 1983). Electrophysiologically, it was recently shown that the effects of *d*-LSD and DOM on rat locus coeruleus firing could be potently blocked with the selective 5-HT$_2$ receptor antagonists ritanserin and LY 53857 (Rasmussen and Aghajanian 1986).

In radioligand binding studies utilizing rat membrane preparations a strong correlation was initially found between phenylisopropylamine hallucinogen 5-HT$_2$ receptor affinity for ^{3}H-ketanserin specific binding and human hallucinogenic potency (Shannon et al. 1984). In competition studies the hallucinogens tested behaved as agonists (i.e., shallow competition curves and a rightward affinity shift in the presence of GTP analogs). No correlation was found between potency and affinity for ^{3}H-LSD-labelled 5-HT$_1$ receptors. More recently, we have shown that the rat brain 5-HT$_2$ receptor can be labelled with ^{3}H-DOB, a known human hallucinogenic agent (Titeler et al. 1985; Lyon et al. 1987) and that hallucinogenic potencies correlated strongly with affinities at ^{3}H-DOB-labelled 5-HT$_2$ receptors (Titeler et al. 1988). We have also shown that a large series of indolealkylamine hallucinogens interact with 5-HT$_2$ receptors with similar properties to the phenylisopropylamine hallucinogens (Lyon et al. 1988). In this study we have extended our investigations to include in vitro radioligand binding and receptor autoradiography of human brain cortical samples in order to determine if a correlation exists between hallucinogenic drug affinities for human 5-HT$_2$ receptors and human potencies.

Materials and methods

Tissue preparation and radioligand binding assays were performed as previously described (Lyon et al. 1987). Briefly, human brain cortices were obtained from the brain

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Table 1. Hallucinogen affinity and potency at brain 5-HT_2 receptors

Compound	Human cortex		Rat cortex		Dose
	K_i	Hill	K_i	Hill	
1) *d*-LSD	0.95± 0.04	0.81±0.05	1.34± 0.3	0.78±0.04	6.70
2) DOI	6 ± 1	0.75±0.04	18.9 ± 2.6[a]	0.73±0.04	5.40
3) (−) DOB	17 ± 1	0.63±0.10	24 ± 3[b]	0.80±0.03	5.80
4) DOET	53 ± 10	0.87±0.05	100 ± 24[c]	ND	4.98
5) (+) DOB	55 ± 12	0.82±0.07	146 ± 9[b]	0.87±0.02	4.80
6) DOM	162 ± 39	0.77±0.04	100 ± 17[a]	0.71±0.04	4.85
7) 5-OMe-DMT	207 ± 34	0.77±0.08	600 ± 40[d]	0.85±0.03	4.55
8) DMT	462 ± 42	1.00±0.09	1200 ± 40[d]	0.86±0.02	3.42
9) 2,5DMA	3000 ±116	0.84±0.05	5200 ± 360[a]	0.85±0.03	3.66
10) iso-DOM	6299 ±118	1.04±0.15	ND	ND	ND
11) MDA	8340 ±616	0.86±0.02	5330 ± 600[e]	ND	3.24
12) MDMA	>10000	ND	8300 ±1100[e]	ND	3.24
13) 3,4,5 TMA	>10000	ND	>10000[e]	ND	3.14

Correlations: human potency, human affinity $r=0.97$; human affinity, rat affinity $r=0.99$

Competition experiments were performed as described in Materials and methods. Values are K_i and Hill coefficients ±SEM. Dose represents the mean human dose (-log moles) and are from Shulgin (1978) and Glennon (1984b). Rat cortical data: [a] (Shannon et al. 1984), [b] (Lyon et al. 1987), [c] (Glennon et al. 1984b), [d] (Lyon et al. 1988), [e] (Lyon et al. 1986). ND is not determined

bank at the Johns Hopkins School of Medicine of patients dying from natural causes. Following autopsy the brains were dissected and frozen at −30° C. Human cortical membranes were prepared by homogenization in an ice-cold buffer containing 50 mM TRIS-HCl, 10 mM $MgSO_4$ and 0.5 mM Na_2 EDTA (pH 7.4 at 37° C). Following homogenization, samples were centrifuged at 30000 *g* for 15 min. The supernatant was discarded and the pellet was resuspended and pre-incubated at 37° C for 15 min. Following centrifugation the samples were resuspended and washed again. Pellets were stored frozen at −30° C until used. The final assay buffer consisted of 50 mM TRIS-HCl, 0.5 mM Na_2 EDTA, 10 mM $MgSO_4$, 10 μM pargyline and 0.1% ascorbate (pH 7.4 at 37° C). 5-HT_2 receptors were labelled with 0.4 nM of the antagonist radioligand ^{3}H-ketanserin (72 Ci/mmol, NEN) in 8 mg/ml wet weight of human cortical tissue. Cinanserin 1 μM was used to define the specific binding. In all assays 10 nM prazosin was included to preclude binding to alpha$_1$ receptors (Battaglia et al. 1983). Since ^{3}H-ketanserin is known to bind with high affinity to a DOPAC release site on monoamine nerve endings, 1 μM tetrabenazine was included in selective assays for comparative purposes (Leysen et al. 1987). Eleven concentrations of competing drug were made fresh daily and the final assay volume was 2.0 ml. Under these conditions ^{3}H-ketanserin binding was 70% specific and amounted to 3000 specific dpm. Samples were incubated for 20 min at 37°C and were then vacuum filtered over glass fiber filters and washed with 10 ml of the experimental buffer. Individual filters were inserted into vials along with 5 ml liquid scintillation fluid (Liquiscint) and equilibrated for 6 h before counting on a Beckman 3801 counter. Displacement data were analyzed using the program EBDA (McPherson 1983) to determine apparent K_i and Hill coefficient values. Other statistical parameters were obtained using the RS1 (BBN) software package.

Drug potencies were also evaluated in frozen sections of human temporal cortex. Cryostat sections (20 mm) were thaw-mounted on gelatin-coated slides and stored at −20° until use. After thawing and drying, sections were covered by 3 ml of a solution consisting of 1.5 nM ^{3}H-ketanserin, 1 μM prazosin and various concentrations of cold competitor in 170 mM Tris buffer, pH 7.7 at 24° C. Non-specific binding was defined by the activity remaining on the section in presence of 1 μM methysergide or 1 μM mianserin. Incubation was conducted for 1 h at room temperature and was ended by two 15-min washes in ice-cold buffer. Methysergide or mianserin displaceable binding was 50% of total binding under these conditions. Tetrabenazine (5 μM) did not inhibit ^{3}H-ketanserin binding and was not included in the assay. Sections were then either wiped with a glass filter and the filters counted as above, or dried with cold air and later applied on Ultrofilm (LKB) for 2 months. Drug potencies on human sections were correlated with drug potencies in membrane assay by linear regression.

Table 2. Effects of tetrabenazine on displacement assays

Compound	K_i	Hill
1) *d*-LSD	0.95±0.04	0.87±0.09
2) (+) DOB	54 ±9	0.76±0.07

Competition experiments were performed as described in Materials and methods. Results are K_i and Hill coefficients ±SEM

Results

The results of the radioligand binding competition experiments are shown in Table 1 and include the K_i and Hill coefficient values for hallucinogenic drug competitions in human cortical homogenates. Also included are the human hallucinogenic potency values for each compound and their affinities at rat brain 5-HT_2 receptors for comparative purposes.

As can be seen from Table 1, *d*-LSD was the most potent compound with a K_i value of 0.95 nM. The potent phenylisopropylamine hallucinogen DOI had an affinity of 6 nM. Stereospecific binding interactions were found with

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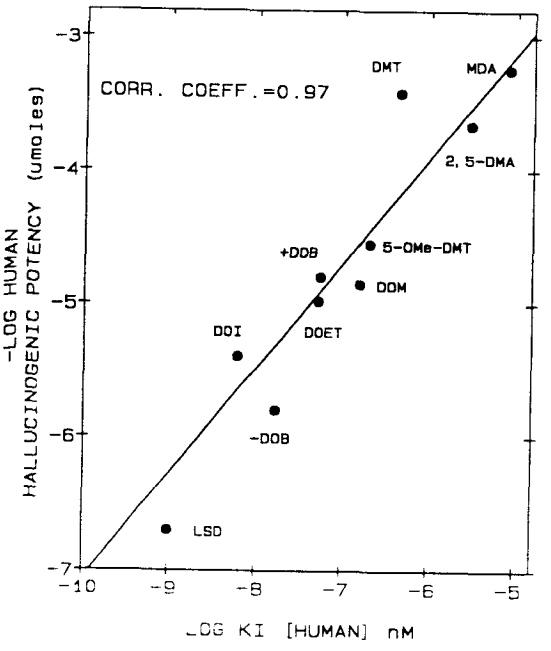

Fig. 1. Correlation between the affinities of ten hallucinogenic agents for ^{3}H-ketanserin-labelled human brain 5-HT$_2$ receptors and their threshold potencies for producing hallucinogenic effects in humans

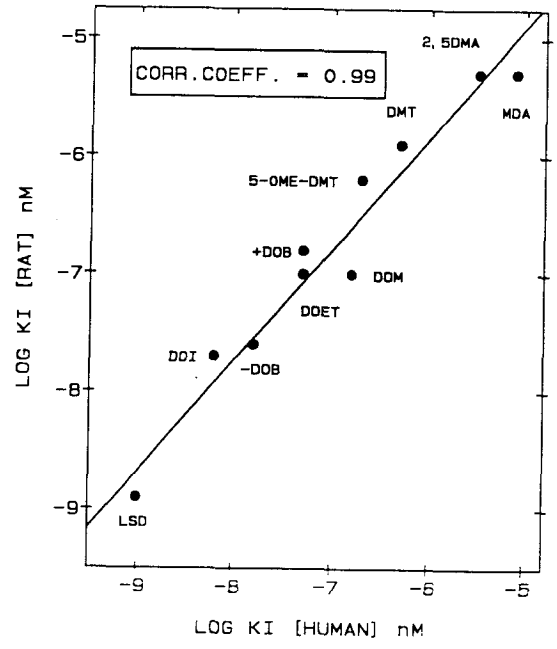

Fig. 2. Correlation between the affinities of ten hallucinogenic agents for ^{3}H-ketanserin-labelled human brain 5-HT$_2$ receptors and ^{3}H-ketanserin-labelled rat brain 5-HT$_2$ receptors

the phenylisopropylamine DOB; the behaviorally active (−) DOB had an affinity of 17 nM while the behaviorally less potent enantiomer (+) DOB had an affinity of 55 nM. Another phenylisopropylamine hallucinogen, DOM, had an affinity of 162 nM while its behaviorally inactive congener iso-DOM had an affinity of 6299 nM. MDA, a compound purported to have both hallucinogenic and stimulant properties (Shulgin 1978), had an affinity constant of 8340 nM while its methylated congener MDMA (Ecstasy) was inactive with a K_i of greater than 10 μM. Subjective reports have stated that ingestion of MDMA is not "hallucinogen like" (Shulgin 1978) and MDMA is not recognized by rats trained with the hallucinogen DOM in drug discrimination procedures (Glennon et al. 1982). The indolealkylamines 5-OMe-DMT and DMT competed with affinities paralleling their human hallucinogenic potencies. Overall, hallucinogenic drug competition for ^{3}H-ketanserin-labelled 5-HT$_2$ receptors was indicative of an agonist interaction (i.e., Hill coefficients were generally significantly less then unity).

^{3}H-Ketanserin has been shown to bind with high affinity to a DOPAC release site on monoamine nerve endings and this site may be blocked selectively with the agent tetrabenazine (Leysen et al. 1987). In assays where 1 μM tetrabenazene was included [*d*-LSD and (+) DOB] no differences were noted in affinity or Hill coefficients (Table 2).

Figure 1 shows the correlation plot of human hallucinogenic potency (mean dose in log μM) versus 5-HT$_2$ receptor affinity in human tissue. A correlation coefficient of $r = 0.97$ was found. A strong correlation ($r = 0.99$) was also found between receptor affinities in rat and human tissue (Fig. 2). Results obtained with human sections are in close agreement with the results obtained in membrane homogenates, which suggests that the rank order of potencies of these hallucinogenic drugs are not significantly influenced by tissue preparation. An excellent correlation ($r = 0.95$) was found between the potencies of drugs tested by these two methods (Fig. 3). The autoradiographs displayed in Fig. 4 demonstrate the specificity of the radiolabelled brain 5-HT$_2$ receptor autoradiographical signal in that mianserin and DOI completely inhibit the binding of ^{3}H-ketanserin in human cortical tissue slices and there is no evidence that ^{3}H-ketanserin labels 5-HT$_{1C}$ receptors (which do have high affinities for DOI and mianserin).

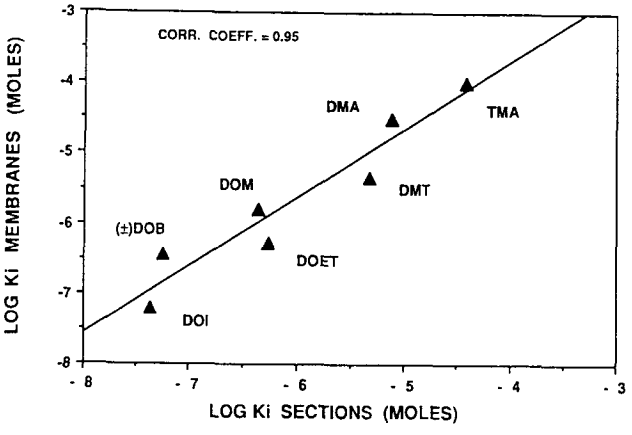

Fig. 3. Correlation between the affinities of seven hallucinogenic agents for ^{3}H-ketanserin-labelled human brain 5-HT$_2$ receptors in tissue homogenate preparations versus their affinities for ^{3}H-ketanserin-labelled human brain 5-HT$_2$ receptors determined using slide-mounted tissue slices (see Materials and methods)

Discussion

The results presented here support the hypothesis that stimulation of human brain 5-HT$_2$ receptors is the mechanism of action of LSD, the phenylisopropylamine and indoleal-

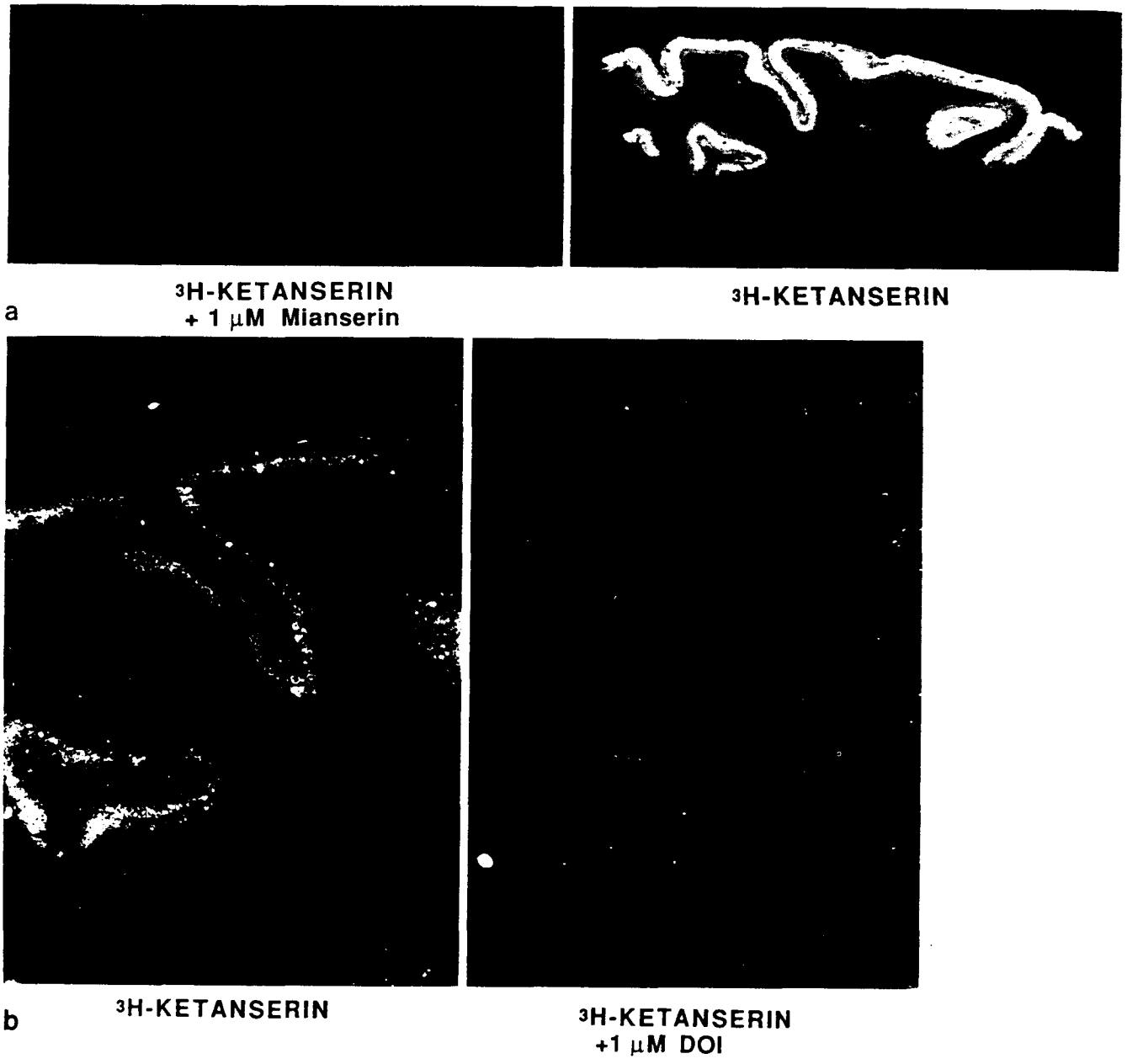

Fig. 4a, b. Autoradiography of human temporal cortex with ^{3}H-ketanserin. There is an intense labelling of the intermediate layers of the cortex. Displacement by mainserin (**a**) and DOI (**b**) indicates the specific labeling of 5-HT_2 receptors

kylamine hallucinogens. These results in human cortex parallel and support our earlier findings in rat cortex where strong correlations were found between hallucinogenic potency and 5-HT_2 receptor affinity (Shannon et al. 1984; Titeler et al. 1988). In earlier studies in rat brain we had used ^{3}H-DOB (Lyon et al. 1987) and ^{125}I-DOI (Glennon et al. 1988) (putative agonist radioligands) to label a guanyl nucleotide sensitive state of the 5-HT_2 receptor. This state exists as a small fraction of the total 5-HT_2 receptor population. Since the density of 5-HT_2 receptors in human brain is much less than that of the rat, these radioligands were not useful for this study. We have also previously shown that hallucinogen competition for ^{3}H-ketanserin-labelled 5-HT_2 receptors is affected by guanine nucleotide analogs, presumably indicative of an agonist interaction with the 5-HT_2 receptor (Shannon et al. 1984; Lyon et al. 1987). In preliminary studies the effects of Gpp(NH)p (a non-hydrolyzable GTP analog) on hallucinogen competition were small and inconsistent (data not shown). This is most probably due to a loss of GTP binding proteins during the pre- and post-autopsy period. It has been recently shown that certain phenylisopropylamines such as DOB (Titeler et al. 1988) and DOI (Hoyer and Karpf 1988) have high affinity for the 5-HT_1C receptor and that a significant correlation exists between hallucinogenic potency and 5-HT_1C receptor binding affinity (Titeler et al. 1988). Due to the lack of a substantial density of these receptors in human cortex, we did not include them in this study. The role of the 5-HT_1C receptor in mediating hallucinogenic effects of drugs is unclear at this time.

In summary, the data presented here demonstrate the potent interaction of LSD, phenylisopropylamine and indo-

lealkylamine hallucinogens with human brain 5-HT_2 receptors. The affinities of these drugs for the human 5-HT_2 receptor strongly correlate with their psychoactive potencies which suggest that their pharmacological properties are mediated through stimulation of these receptors. Accordingly, 5-HT_2 receptor antagonists should prove to be useful in treating the psychiatric symptoms secondary to ingestion of LSD and other hallucinogens.

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