Antagonism of 5-Hydroxytryptamine$_2$ Receptor-Mediated Phosphatidylinositol Turnover by *d*-Lysergic Acid Diethylamide[1]

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ABSTRACT

The interactions of the indolealkylamine hallucinogen *d*-lysergic acid diethylamide (*d*-LSD) and two phenalkylamine hallucinogens, 2,5-dimethoxy-4-bromoamphetamine (DOB) and 2,5-dimethoxy-4-iodoamphetamine (DOI), with 5-hydroxytryptamine$_2$ (5-HT$_2$) receptors were analyzed in rat cortex using both radioligand binding techniques and biochemical measurements of phosphatidylinositol (PI) turnover. 5-HT$_2$ binding sites were labeled by [^{3}H]ketanserin. DOB and DOI displayed decreased affinity for 5-HT$_2$ sites in the presence of 10^{-4} M GTP, whereas the ability of *d*-LSD to compete for these sites was not affected by the presence of 10^{-4} M GTP. Moreover, the Hill slope of the *d*-LSD competition curve was unity in both the absence and presence of 10^{-4} M GTP. These findings suggest that *d*-LSD is an antagonist at 5-HT$_2$ receptors. PI turnover studies in rat cortex showed that at 10^{-5} M concentrations *d*-LSD, DOB and DOI display partial agonist activity in comparison to 10^{-5} 5-HT. Stimulation of PI turnover by 5-HT, DOB and DOI was inhibited by the 5-HT$_2$ antagonist ketanserin (10^{-6} M). The *d*-LSD PI signal was not affected by the presence of ketanserin. In addition, nanomolar concentrations of *d*-LSD did not stimulate PI turnover in rat cortex. Moreover, nanomolar concentrations of *d*-LSD are able to significantly antagonize the stimulatory effect of 10^{-5} M 5-HT on PI turnover. These data suggest that *d*-LSD acts as an antagonist at 5-HT$_2$ receptors in rat cortex. At high concentrations (>1 μM) *d*-LSD stimulates low-level PI turnover *via* a non-5-HT$_2$ receptor-mediated mechanism.

Since the discovery in 1938 of the hallucinogenic agent *d*-LSD, many attempts have been made to identify the mechanism of action by which hallucinogens exert their effects on the central nervous system. A variety of data indicates that hallucinogens modulate serotonergic neurotransmission. For example, 5-HT as well as certain hallucinogens potently inhibit raphe cell firing (Aghajanian *et al.*, 1987). Hallocinogens also have a number of behavioral effects which appear to be mediated by their effects on 5-HT neurotransmission (Jacobs, 1987). However, the exact relationship between hallucinogenic effects and 5-HT neurotransmission has remained obscure.

Classic hallucinogens include two major structural categories: indolealkylamines such as *d*-LSD and phenalkylamines such as DOB and DOI. Glennon and associates have hypothesized recently that these hallucinogens act as direct agonists at the 5-HT$_2$ receptor in the central nervous system (Glennon, 1985; Glennon *et al.*, 1986). This theory is based largely on two observations. First, the affinity of hallucinogenic agents for 5-HT$_2$ binding sites correlates significantly with their human potencies (Glennon *et al.*, 1984; Titeler *et al.*, 1988). Second, *d*-LSD and other hallucinogens produce similar stimulus effects in drug discrimination studies (Glennon *et al.*, 1986; Appel and Cunningham, 1986). Moreover, specific 5-HT$_2$ antagonists are able to block these discriminative cue effects (Glennon *et al.*, 1983; Colpaert *et al.*, 1985; Nielsen *et al.*, 1985). Therefore, at least certain central nervous system effects of hallucinogens may be specifically mediated by 5-HT$_2$ receptors.

The 5-HT$_2$ receptor, along with the 5-HT$_{1C}$ receptor, has also been linked to the PI second-messenger system (Sanders-Bush and Conn, 1987). 5-HT stimulates PI turnover with an EC$_{50}$ value of 1 μM (Conn and Sanders-Bush, 1985). Stimulation of PI turnover by 5-HT in rat cerebral cortex is potently blocked by selective 5-HT$_2$ antagonists (Conn and Sanders-Bush, 1984; Kendall and Nahorski, 1985). In addition, chronic antidepressant treatment decreases both 5-HT$_2$ radioligand binding and the cortical response to 5-HT (Kendall and Nahorski, 1985).

Therefore, the 5-HT$_2$ receptor has been linked to both the agonist effects of hallucinogens and stimulation of PI turnover in the rat central nervous system. The present study attempted to determine if *d*-LSD acts as an agonist at 5-HT$_2$ receptors by analyzing both the effect of GTP on *d*-LSD interactions with binding sites labeled by [^{3}H]ketanserin and the effect of *d*-LSD on 5-HT$_2$ receptor-mediated PI turnover in rat cortex.

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ABBREVIATIONS: *d*-LSD, *d*-lysergic acid diethylamide; 5-HT, 5-hydroxytryptamine; DOB, 2,5-dimethoxy-4-bromoamphetamine; DOI, 2,5-dimethoxy-4-iodoamphetamine; PI, phosphatidylinositol.

Materials and Methods

Radioligand studies. Receptor binding assays were performed according to the methods of Leysen *et al.* (1982). Briefly, adult rat brains were obtained immediately after decapitation and the frontal cortex dissected as needed. Tissues were homogenized in 20 volumes of 50 mM Tris-HCl buffer (pH 7.7 at 25°C) using a Brinkmann Polytron and then centrifuged in an IEC B20A centrifuge at 49,000 × *g* for 10 min. The supernatant was discarded and the pellet resuspended in the same volume of Tris-HCl buffer and incubated at 37°C for 10 min before a second centrifugation at 49,000 × *g* for 10 min. The final pellet was resuspended in 80 volumes of Tris-HCl buffer containing 10 μM pargyline, 4 mM calcium chloride and 0.1% ascorbic acid. The suspensions were used immediately in the binding assay.

Binding assays for drug inhibition studies consisted of 0.1 ml of [^{3}H]ketanserin (final concentration of 0.4–0.8 nM), 0.1 ml of buffer or competing drug and 0.8 ml of tissue suspension. After incubation at 25°C for 30 min, the assays were filtered rapidly under vacuum through Whatman GF/B filters with two 5-ml washes using 50 mM Tris-HCl buffer. Radioactivity was measured by liquid scintillation spectroscopy in 5 ml of Aquasol (New England Nuclear, Boston, MA) at approximately 54% efficiency. Specific binding was defined using 1 μM cinanserin in all experiments.

PI turnover. The methods used in this study to determine PI turnover in cortical slices are modified slightly from the methods of Berridge *et al.* (1982). Cortices were dissected from Sprague-Dawley rats (100–200 g) and cut into 350 × 350 μm slices using a Brinkmann McIlwain Tissue Chopper. Slices were then incubated at 37°C for 45 min in a shaking water bath with modified Krebs' solution and bubbled O_2-CO_2 (95%-5%). Aliquots of slices (50 μl) were transferred to scintillation vials along with 1 μCi of [^{3}H]myoinositol, 6 mM lithium chloride and 10 μM pargyline in modified Krebs' buffer. Vials were then gassed, capped and incubated again for 45 min at 37°C. After this time antagonists were added if indicated and incubated for 15 min. Agonists were then added, the vials were again gassed, capped and incubated at 37°C for 45 min. A chloroform/methanol mixture (1:2 v/v) was added to each vial to stop the reaction. Distilled H_2O and $CHCl_3$ were added to each vial in order to separate the solution into phases. The vials were then vortexed and set aside to allow phase separation.

Dowex ion-exchange columns (200–400 mesh) were used for the extraction. The aqueous phase from each vial was transferred to the columns. Two washes were performed with 5 mM unlabeled myoinositol. When the washes were complete, collection tubes were set up and the radiolabeled inositol phosphates were eluted with 1 N ammonium formate in 0.1 N formic acid. From each tube, the eluate was transferred to scintillation vials along with 10 ml of Aquasol scintillation cocktail. The vials were then placed in a liquid scintillation counter. The cpm of radioactivity obtained from drug-stimulated tissue were divided by the cpm values obtained from unstimulated control tissue so that the levels of PI turnover are reported as a percentage above the control response. The unpaired Student's *t* test was used to determine the level of significance between mean assay values.

Drugs were obtained from the following sources: [^{3}H]ketanserin (61.8 Ci/mmol; New England Nuclear, Boston, MA); [^{3}H]myoinositol (15 Ci/mmol; American Radiolabeled Chemicals Inc., St. Louis, MO); 5-HT, GTP (Sigma Chemical Co., St. Louis, MO): *d*-LSD, DOI and DOB (National Institute on Drug Abuse, Bethesda, MD); cinanserin (E. R. Squibb & Sons, Inc., Princeton, NJ) and ketanserin (Janssen R & D, Inc., New Brunswick, NJ).

Results

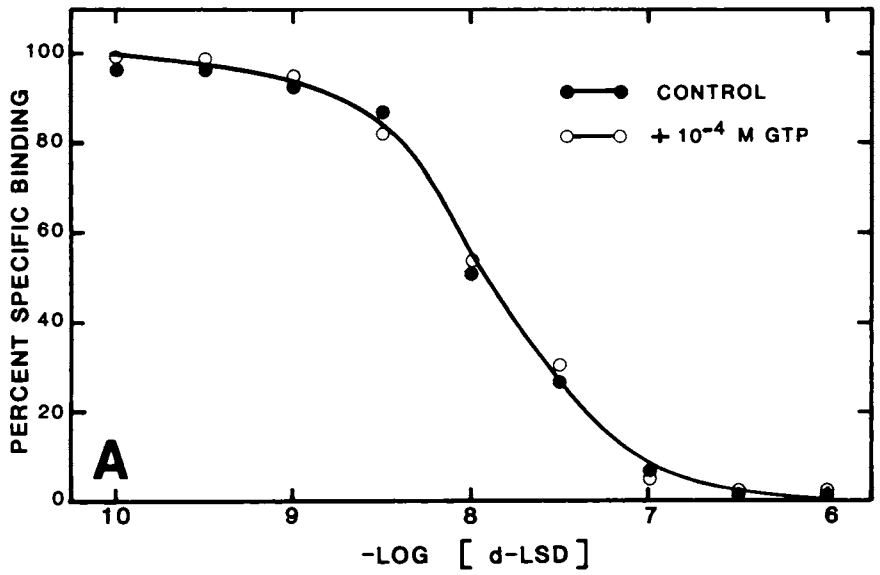
Effect of 10^{-4} M GTP on *d*-LSD, DOB and DOI competition for 5-HT_2 receptors labeled by [^{3}H]ketanserin in rat frontal cortex. Drug competition studies with *d*-LSD, DOB and DOI were performed with [^{3}H]ketanserin in rat frontal cortex in the absence or presence of 10^{-4} M GTP. This nucleotide concentration has been reported to decrease the potency of agonists, but not antagonists, at the 5-HT_2 binding site (Battaglia *et al.*, 1984). As shown in figure 1A, the ability of *d*-LSD to compete for specific [^{3}H]ketanserin is essentially identical in the absence or presence of 10^{-4} M GTP. *d*-LSD begins to inhibit specific [^{3}H]ketanserin binding at concentrations above 10^{-9} M. The IC_{50} is approximately 10^{-8} M. Specific [^{3}H]ketanserin binding is eliminated by *d*-LSD concentrations above 3×10^{-7} M. The competition curves are monophasic with Hill coefficients of 1.07 ± 0.05 in the absence and 1.00 ± 0.04 in the presence of 10^{-4} M GTP. The apparent affinity constant (K_i value) of *d*-LSD *vs.* [^{3}H]ketanserin binding in rat cortex is 4.7 ± 1 nM in the absence and 4.4 ± 0.7 nM in the presence of 10^{-4} M GTP. Therefore, 10^{-4} M GTP has no significant effect on either the Hill slope or K_i value of *d*-LSD *vs.* [^{3}H]ketanserin in rat cortex.

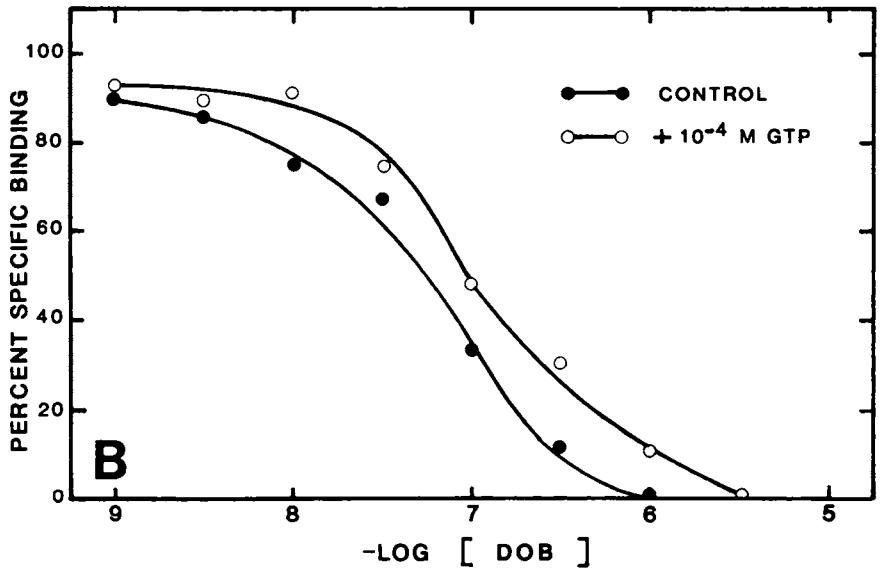
In contrast, 10^{-4} M GTP significantly decreases the affinity of DOB and DOI for the [^{3}H]ketanserin-labeled sites. The data from a competition experiment with DOB and DOI are shown in figure 1, B and C, respectively. Without GTP, the IC_{50} for DOB is approximately 40 nM. The addition of 10^{-4} M GTP increases the IC_{50} for DOB to 90 nM. The K_i value of DOB *vs.* [^{3}H]ketanserin binding in rat cortex averages 44 ± 9 nM in the absence and 92 ± 20 nM in the presence of 10^{-4} M GTP ($P < .005$; *t* test). Similarly, the K_i value of DOI *vs.* [^{3}H]ketanserin binding averages 33 ± 3 nM in the absence and 55 ± 7 nM in the presence of 10^{-4} M GTP ($P < .01$; *t* test).

Effect of 5-HT on PI turnover in rat cortex. Rat cortical slices were incubated with increasing concentrations of 5-HT for 45 min. A typical dose-response curve for 5-HT-stimulated PI turnover is shown in figure 2. A dose-dependent increase in PI stimulation above base-line values is observed with 5-HT concentrations between 10^{-8} and 10^{-5} M. The EC_{50} value for 5-HT in this system is approximately 1 μM, in agreement with the value obtained by other investigators (Conn and Sanders-Bush, 1985). Stimulation of PI turnover plateaus between 10^{-5} and 10^{-4} M 5-HT. The mean basal rate of PI turnover (control) for all assays performed in this study was 1630 ± 100 cpm.

Effect of 10^{-5} M *d*-LSD, DOB and DOI on PI turnover in rat cortex. Rat cortical slices were incubated for 45 min in the presence of 10^{-5} M 5-HT, *d*-LSD, DOB or DOI (fig. 3). The PI turnover level obtained with 10^{-5} M 5-HT averaged 62 ± 3% above base-line levels (designated as 100% of the 5-HT response in fig. 3). In the presence of 10^{-5} M *d*-LSD, PI turnover was stimulated to 20 ± 7% of the 10^{-5} M 5-HT response. DOB (10^{-5} M) stimulated PI turnover to 35 ± 6% of the 5-HT response. DOI produced the largest PI stimulation of the three hallucinogenic agents tested, as evidenced by its ability to increase PI turnover to 48 ± 8% of the 5-HT response.

Effect of 10^{-6} M ketanserin on PI turnover stimulated by 10^{-5} M concentrations of 5-HT, *d*-LSD, DOB and DOI. In order to determine if the PI stimulating effect of 5-HT and the three hallucinogens is *via* the 5-HT_2 receptor, the selective 5-HT_2 antagonist ketanserin (10^{-6} M) was added 15 min before the addition of these compounds (fig. 4). The PI signal obtained with 10^{-5} M 5-HT averaged 155 ± 3% above base-line levels and is designated as 100% response. The presence of 10^{-6} M ketanserin significantly reduced the 5-HT response from 100 ± 5% to 12 ± 5% ($P < .001$; *t* test). The *d*-LSD PI signal was not affected by the presence of ketanserin, with a level of 27 ± 4% of the 5-HT signal in the absence and 24 ± 3% of the 5-HT signal in the presence of 10^{-6} M ketanserin. By contrast, the DOB PI signal was reduced significantly





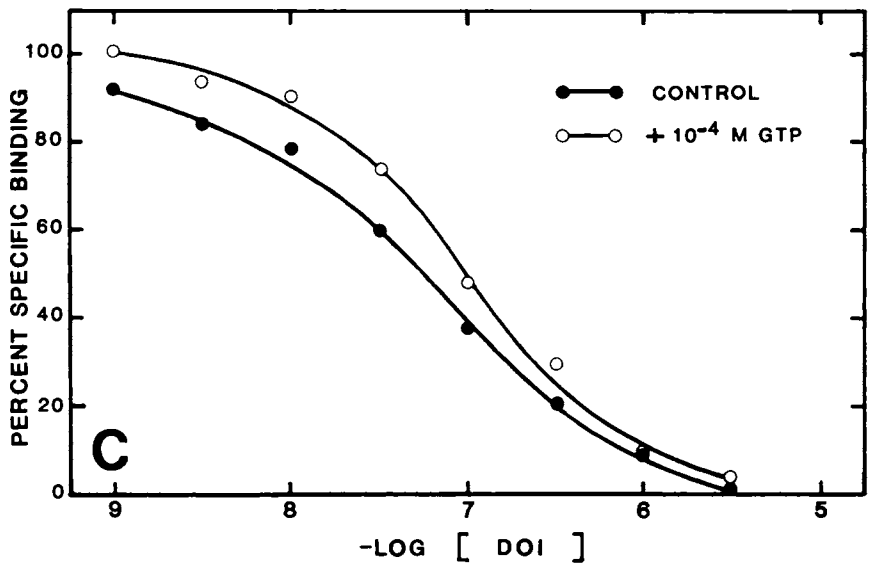


Fig. 1. Effect of 10^{-4} M GTP on (A) *d*-LSD (B) DOB and (C) DOI competition for 5-HT_2 receptors labeled by [^{3}H]ketanserin in rat frontal cortex. Radioligand binding studies were performed as described under "Materials and Methods." A final concentration of 0.4 to 0.8 nM [^{3}H]ketanserin was used in the assay. Specific binding for [^{3}H]ketanserin was defined as the excess over blanks taken in the presence of 1 μM cinanserin. The data shown are the results of a single experiment, performed in triplicate. Each experiment was repeated three times.

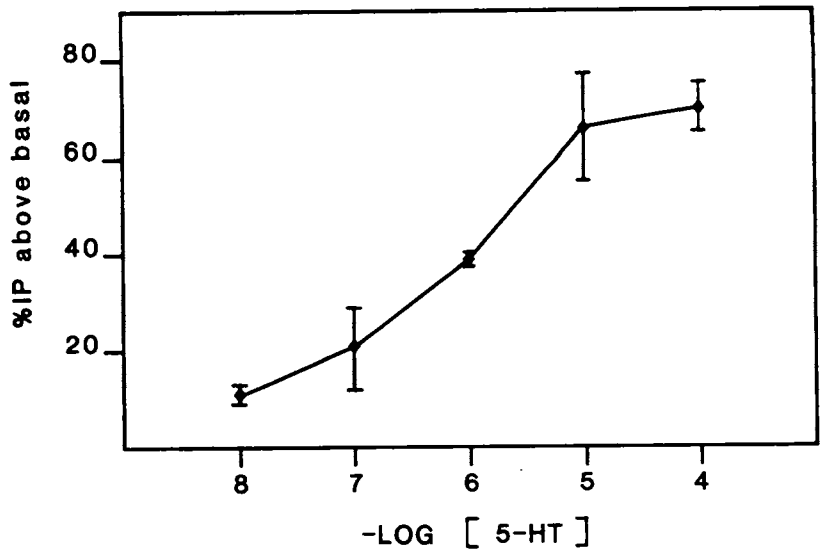


Fig. 2. Effect of 5-HT on inositol phosphate formation in the rat cortex. PI turnover experiments were performed as described under "Materials and Methods." 5-HT was incubated with the slices for 45 min. The data given are the percentage response ± S.E.M. above base-line levels of inositol phosphate production. The data shown are from a single experiment, performed in triplicate.

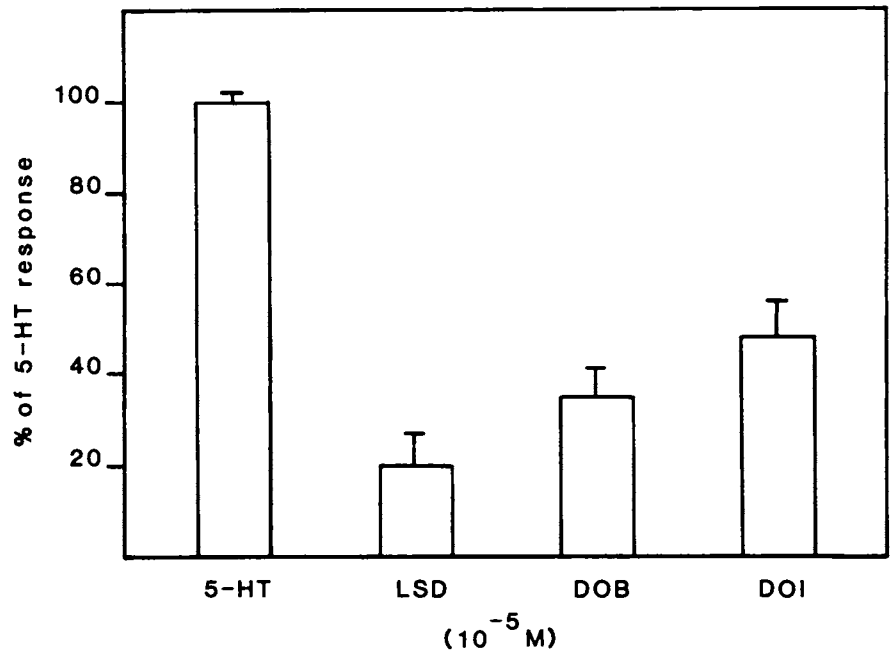


Fig. 3. Drug effects on PI turnover in rat cortex. Inositol phosphate levels were measured in rat cortex after a 45-min incubation with 10^{-5} M concentrations of 5-HT, *d*-LSD, DOB or DOI. The 5-HT PI signal averaged 62 ± 3% above base-line levels and is represented as 100% for 5-HT. Hallucinogen effects are shown as a percentage of the 10^{-5} M 5-HT response. Data are from 3 to 18 separate experiments, each performed in at least triplicate. Vertical bars represent S.E.M.

from 39 ± 3% of the 5-HT signal to −4 ± 3% of the 5-HT signal in the presence of ketanserin ($P < .002$; *t* test). Similarly, the DOI PI signal was reduced significantly from 46 ± 5% of the 5-HT signal to 5 ± 3% of the 5-HT signal in the presence of ketanserin ($P < .002$; *t* test).

Effect of 10^{-5} M *d*-LSD, DOB and DOI on 10^{-5} M 5-HT-stimulated PI turnover in rat cortex. The ability of the three hallucinogenic agents to modulate PI turnover was then analyzed in the presence of 5-HT (fig. 5). Each of the hallucinogens was added to the assay 15 min before the addition of 10^{-5} M 5-HT. The control response (fig. 4) represents the amount of PI stimulation obtained with 10^{-5} M 5-HT in the absence of hallucinogens. A concentration of 10^{-5} M *d*-LSD reduced significantly the 5-HT signal to 34 ± 10% of the control response ($P < .001$; *t* test). DOB also produced a significant reduction in the 5-HT signal to 82 ± 4% of the control response ($P < .05$; *t* test). DOI reduced slightly the PI signal to 89 ± 8% of the 5-HT control response.

Effect of *d*-LSD on 5-HT-stimulated PI turnover in rat cortex. In order to characterize further the effect of *d*-LSD on 5-HT-stimulated PI turnover, *d*-LSD dose-response curves were analyzed in the absence and presence of 10^{-5} M 5-HT. As shown in figure 6, in the absence of 5-HT, essentially no PI stimulation occurs at *d*-LSD concentrations below 10^{-6} M. PI stimulation by *d*-LSD at concentrations above 10^{-6} M is approximately 20% of the 5-HT signal.

By contrast, nanomolar concentrations of *d*-LSD have significant effects on PI turnover when examined in the presence of 10^{-5} M 5-HT. As shown in figure 6, a reduction in the stimulatory effect of 10^{-5} M 5-HT on PI levels occurs at a *d*-LSD concentration of 1 nM (71 ± 10%). The IC_{50} value for *d*-LSD in this system is approximately 10 nM. The inhibitory effect of *d*-LSD on the 5-HT-stimulated PI turnover appears to plateau at concentrations above 100 nM *d*-LSD. Approximately 35% of the 10^{-5} M 5-HT response is observed at *d*-LSD concentrations between 10^{-7} and 10^{-5} M.

Discussion

The major finding of the present study is that nanomolar concentrations of *d*-LSD act as an antagonist of 5-HT_2 recep-

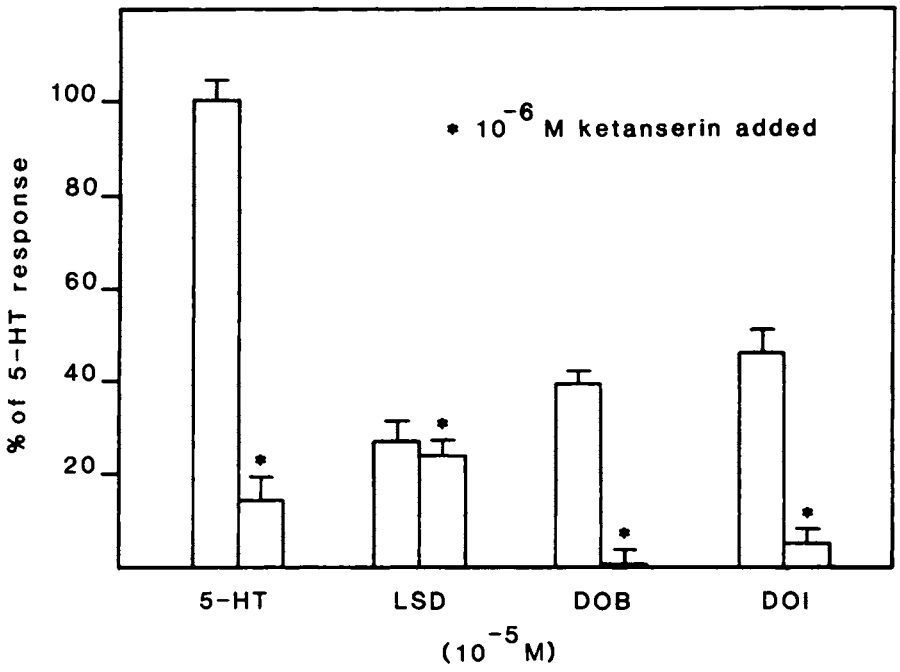


Fig. 4. Effects of 10^{-6} M ketanserin on 5-HT and hallucinogen stimulation of PI turnover in rat cortex. Inositol phosphate levels were measured in rat cortex with and without a prior 15-min incubation with 10^{-6} M ketanserin. The 5-HT PI signal averaged 55 ± 5% above base-line levels and is represented as 100% for 5-HT. Hallucinogen effects are shown as a percentage of the 10^{-5} M 5-HT response. Data are from 3 to 4 separate experiments, each performed in at least triplicate. Vertical bars represent S.E.M.

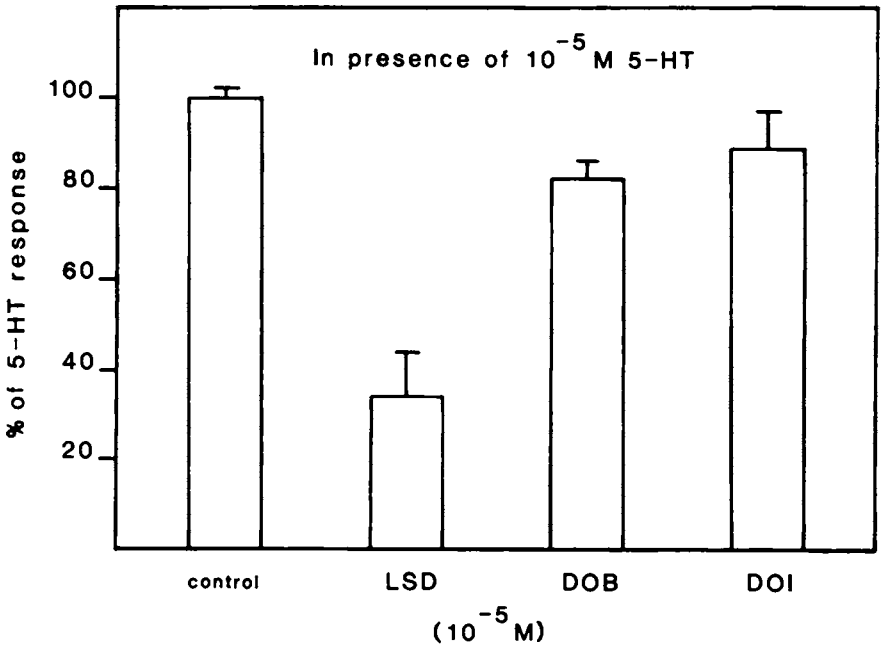


Fig. 5. Drug effects on 10^{-5} M 5-HT-stimulated PI turnover in rat cortex. PI turnover experiments were performed as described under "Materials and Methods." Data shown are given as the percentage ± S.E.M. of the inositol phosphate levels obtained with 10^{-5} M5-HT alone (shown as control condition). Data are from three separate experiments, each performed in triplicate.

tors in rat cortex. This conclusion is based on the two major observations in the present study. First, GTP fails to alter the ability of *d*-LSD to compete for 5-HT_2 receptors, a finding which suggests a lack of agonist activity at this site (Battaglia *et al.*, 1984). Second, nanomolar concentrations of *d*-LSD significantly inhibit the effects of micromolar 5-HT on PI turnover in the rat cortex, a biochemical system mediated by 5-HT_2 receptors (Sanders-Bush and Conn, 1987; Kendall and Nahorski, 1985). High concentrations of *d*-LSD (>1 μM) stimulate low levels of PI turnover; however, this effect is not blocked by the 5-HT_2 antagonist ketanserin (10^{-6} M). The possibility that *d*-LSD might act as a very weak partial agonist at 5-HT_2 receptors and therefore would appear as an antagonist in the presence of 5-HT must be considered. However, the fact that PI stimulation by *d*-LSD is ketanserin-insensitive argues against this possibility.

The phenalkylamine hallucinogens, DOB and DOI, appear to be partial agonists at the 5-HT_2 receptor in rat cortex. The apparent K_i values for DOB and DOI *vs.* [^{3}H]ketanserin binding are increased 2-fold and 1.7-fold, respectively, in the presence of 10^{-4} M GTP. This finding suggests agonist activity of these compounds at the [^{3}H]ketanserin site (Battaglia *et al.*, 1984). In addition, DOB and DOI (10^{-5} M) stimulate PI turnover to 35% and 48% of the 5-HT-stimulated PI level. The PI signal produced by DOB and DOI, as well as 5-HT, is inhibited by 10^{-6} M ketanserin. In addition, DOB and DOI do inhibit 5-HT-induced PI turnover, but this inhibition plateaus at a PI level ranging between 5-HT and the phenalkylamines' agonist abilities.

These data appear to contradict the hypothesis of Glennon and colleagues which states that hallucinogenic drugs mediate

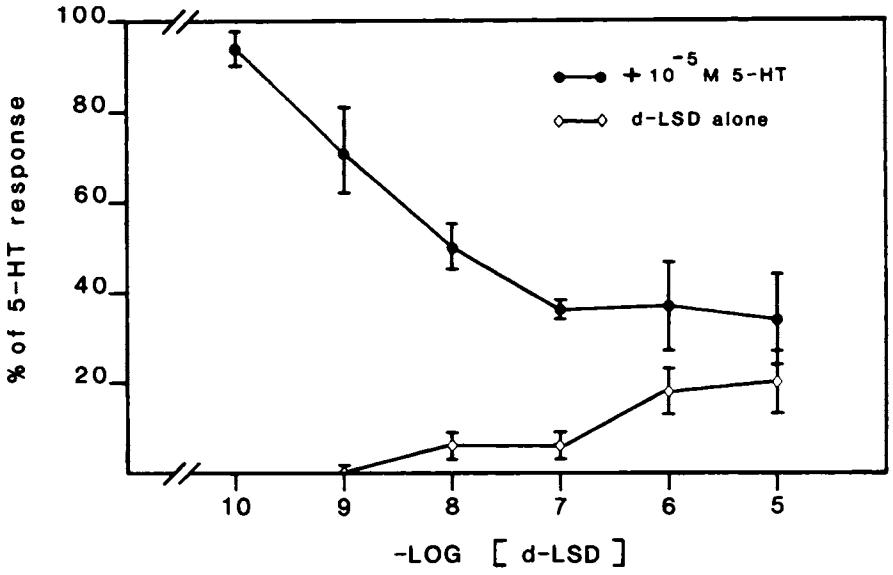


Fig. 6. Effect of increasing concentrations of *d*-LSD on PI turnover in the absence and presence of 10^{-5} M 5-HT. PI turnover experiments were performed as described under "Materials and Methods." Inositol phosphate levels are given as the percentage of the PI response to 10^{-5} M 5-HT alone. Data are from a minimum of three separate experiments performed in triplicate. Vertical bars represent S.E.M.

hallucinosis by acting as agonists at 5-HT_2 receptors (Glennon, 1985; Glennon *et al.*, 1986). Their suggestion has been based on two major findings. First, hallucinogenic potencies in humans correlate with hallucinogenic affinities for 5-HT_2 binding sites (Glennon *et al.*, 1984; Titeler *et al.*, 1988). Second, Glennon and colleagues have pointed out that *d*-LSD and other hallucinogens produce similar stimulus effects in drug discrimination studies (Glennon *et al.*, 1986). Moreover, specific 5-HT_2 antagonists are able to block these discriminative cue effects. However, quipazine (Parati *et al.*, 1980; Friedman *et al.*, 1984; Cunningham and Appel, 1987), lisuride (Mokler *et al.*, 1983) and yohimbine (Colpaert, 1984) are examples of drugs which also generalize to *d*-LSD in discriminative cue studies yet do not produce hallucinations in humans. Because *d*-LSD has been reported to interact with multiple receptors, both serotonergic and nonserotonergic (Whitaker and Seeman, 1977; Peroutka, 1986; unpublished observations), it is not surprising that many drugs may generalize with *d*-LSD. In addition, spiperone does not block the *d*-LSD cue despite the fact that it is an extremely potent 5-HT_2 antagonist (Cunningham and Appel, 1987). Therefore, discriminative cue studies may not be a definitive model of *d*-LSD activation and/or antagonism of the 5-HT_2 receptor.

The hypothesis that hallucinogenic drugs act as agonists at the 5-HT_2 receptor has been tested in the present study using molecular pharmacologic and biochemical techniques. For example, Titeler and colleagues (Battaglia *et al.*, 1984; Lyon *et al.*, 1987) have suggested that [^{3}H]ketanserin labels two "states" of the 5-HT_2 receptor. This conclusion was based on the fact that agonists, but not antagonists, displayed shallow Hill slopes against [^{3}H]ketanserin-labeled 5-HT_2 sites in rat cortex. Moreover, the high affinity or agonist state of the receptor could be eliminated by the addition of 10^{-4} M GTP to the radioligand assay (Battaglia *et al.*, 1984). Thus, 10^{-4} M GTP was reported to decrease the affinity of agonists, but not antagonists, for [^{3}H]ketanserin-labeled sites. In the present study, *d*-LSD displayed a Hill coefficient of 1.07 ± 0.05 *vs.* [^{3}H]ketanserin. In addition, 10^{-4} M GTP had no effect on the ability of *d*-LSD to compete for [^{3}H]ketanserin-labeled sites. According to the hypothesis of Titeler and colleagues, these results indicate that *d*-LSD is an antagonist at 5-HT_2 receptors.

The PI turnover studies in the present report also indicate that *d*-LSD is an antagonist at 5-HT_2 receptors. The extensive work of Conn and Sanders-Bush (1984, 1985, 1986; Sanders-Bush and Conn, 1987) and Kendall and Nahorski (1985) have demonstrated convincingly that 5-HT-stimulated PI turnover is mediated by the 5-HT_2 receptor in rat cortex. Although a large number of both agonists and antagonists have been studied, the present report is the first to analyze hallucinogens in this system. As shown in figure 6, nanomolar concentrations of *d*-LSD fail to stimulate directly PI turnover, yet this concentration of *d*-LSD significantly inhibits the stimulatory effect of 10^{-5} M 5-HT. The ability of nanomolar concentrations of *d*-LSD to antagonize the effect of a 1000-fold higher concentration of 5-HT is consistent with a 5-HT_2-mediated effect, inasmuch as *d*-LSD displays nanomolar affinity and 5-HT displays micromolar affinity for the 5-HT_2 receptor. This effect is not consistent with a 5-HT_{1A}-, 5-HT_{1B}-, 5-HT_{1C}- or 5-HT_{1D}-mediated effect because, in each of these systems, 5-HT is either equipotent or more potent than *d*-LSD (Peroutka, 1986; Heuring and Peroutka, 1987). In addition, the low-level stimulation of PI turnover found at *d*-LSD concentrations above 10^{-6} M is not blocked by 10^{-6} M ketanserin, indicating that micromolar

TABLE 1

Summary of systems in which *d*-LSD antagonizes 5-HT_2-mediated effects

System	References
PI turnover	This report
Guinea pig trachea	Van Nueten *et al.*, 1982; Cohen *et al.*, 1985; Heller and Baraban, 1987
Rat uterus	Gaddum *et al.*, 1955; Cerletti and Doepfner, 1958; Hashimoto *et al.*, 1977; Millar *et al.*, 1982; Cohen *et al.*, 1985
Rat paw edema	Doepfner and Cerletti, 1958; Ortmann *et al.*, 1982
Tryptamine seizures	Tedeschi *et al.*, 1959; Leysen *et al.*, 1978
Head-twitch	Corne *et al.*, 1963; Gerber *et al.*, 1985

concentrations of *d*-LSD are stimulating PI turnover *via* a non-5-HT_2 receptor-mediated mechanism.

The ability of *d*-LSD to antagonize 5-HT_2-mediated effects of 5-HT has also been reported in multiple other systems (table 1). For example, the guinea pig trachea contracts in response to micromolar concentrations of 5-HT (Heller and Baraban, 1987). The ability of 5-HT antagonists to block this effect correlates significantly with drug affinities for the 5-HT_2 binding site (Van Nueten *et al.*, 1982; Cohen *et al.*, 1985). DOB acts as a partial agonist in this system by producing approximately 50% of the maximal contraction induced by 5-HT (Heller and Baraban, 1987). By contrast, nanomolar concentrations of *d*-LSD displayed no agonist effects in this system yet were able to antagonize the effect of micromolar 5-HT. These data are extremely analogous to the PI turnover data described in the present report and further confirm the ability of *d*-LSD to antagonize 5-HT_2 receptor-mediated events.

The rat uterus is a second example of smooth muscle in which 5-HT-induced contractions appear to be mediated by 5-HT_2 receptors (Millar *et al.*, 1982; Cohen *et al.*, 1985). Once again, *d*-LSD acts as an antagonist in this system, with a pA_2 value of approximately 8.5 (Gaddum *et al.*, 1955; Cerletti and Doepfner, 1958; Hashimoto *et al.*, 1977). 5-HT-induced paw edema in rats has also been linked to the 5-HT_2 receptor (Ortmann *et al.*, 1982). The effect of 5-HT in this model system is also antagonized by *d*-LSD (Doepfner and Cerletti, 1958). In addition, the affinity of agonists for the 5-HT_2 receptor in rat cortex labeled with [^{3}H]ketanserin has been shown to be pH dependent, whereas the affinity of antagonists, including *d*-LSD, was not affected by pH (Battaglia *et al.*, 1983).

Behavioral data provide additional evidence for the ability of *d*-LSD to antagonize 5-HT_2 receptors. For example, prevention of tryptamine-induced seizures was the first behavioral model which was correlated with blockade of 5-HT_2 binding sites (Leysen *et al.*, 1978). In this system, *d*-LSD acts as an antagonist (Tedeschi *et al.*, 1959). 5-Hydroxytryptophan-induced head-twitches also appear to be mediated by 5-HT_2 receptors (Peroutka *et al.*, 1981). Although *d*-LSD has been reported to induce head-twitches (Silva and Calil, 1975), it has also been demonstrated to antagonize the head-twitch response to 5-HT stimulation in both mice (Corne *et al.*, 1963) and rats (Gerber *et al.*, 1985).

In summary, the present study presents indirect data from radioligand binding studies and direct data from PI turnover experiments to demonstrate that *d*-LSD acts as an antagonist at 5-HT_2 receptors. Moreover, this conclusion is supported by a review of previous results from multiple other systems in which 5-HT_2 receptors have been analyzed. Although 5-HT_2 receptors may play an important role in the mechanism of action of hallucinogenic agents, these observations suggest that hallucinogenic drug activity is not derived from direct activation of 5-HT_2 receptors.

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