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(+)Lysergic Acid Diethylamide, but not Its Nonhallucinogenic Congeners, Is a Potent Serotonin $5HT_{1C}$ Receptor Agonist[1]

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ABSTRACT

Activation of central serotonin $5HT_2$ receptors is believed to be the primary mechanism whereby lysergic acid diethylamide (LSD) and other hallucinogens induce psychoactive effects. This hypothesis is based on extensive radioligand binding and electrophysiological and behavioral studies in laboratory animals. However, the pharmacological profiles of $5HT_2$ and $5HT_{1C}$ receptors are similar, making it difficult to distinguish between effects due to activation of one or the other receptor. For this reason, it was of interest to investigate the interaction of LSD with $5HT_{1C}$ receptors. Agonist-stimulated phosphoinositide hydrolysis in rat choroid plexus was used as a direct measure of $5HT_{1C}$ receptor activation. (+)LSD potently stimulated phosphoinositide hydrolysis in intact choroid plexus and in cultures of choroid plexus epithelial cells, with EC_{50} values of 9 and 26 nM, respectively. The effect of (+)LSD in both systems was blocked by 5HT receptor antagonists with an order of activity consistent with interaction at $5HT_{1C}$ receptors. Neither (+)-2-bromo-LSD nor lisuride, two nonhallucinogenic congeners of LSD, were able to stimulate $5HT_{1C}$ receptors in cultured cells or intact choroid plexus. In contrast, lisuride, like (+)LSD, is a partial agonist at $5HT_2$ receptors in cerebral cortex slices and in NIH 3T3 cells transfected with $5HT_2$ receptor cDNA. The present finding that (+)LSD, but not its nonhallucinogenic congeners, is a $5HT_{1C}$ receptor agonist suggests a possible role for these receptors in mediating the psychoactive effects of LSD.

The mechanisms by which LSD and other hallucinogens induce their psychoactive effects remain a mystery. Historically, the neuronal serotonergic system has been implicated as playing a major role in the mechanism of action of LSD (for a review see Jacobs and Trulson, 1979; Jacobs, 1987). Recent studies have focused on the 5HT receptor subtype(s) at which LSD induces its primary action. These studies are complicated by the large number of central 5HT receptor subtypes. Currently, at least six 5HT receptor subtypes have been demonstrated in mammalian brain, including $5HT_{1A}$, $5HT_{1B}$, $5HT_{1C}$, $5HT_{1D}$, $5HT_2$ and $5HT_3$ (Schmidt and Peroutka, 1989). LSD binds to several 5HT receptor subtypes as well as dopaminergic and *alpha* adrenergic receptors (Schmidt and Peroutka, 1989; Freedman and Boggan, 1982). Despite this nonspecificity, a growing body of electrophysiological, behavioral and radioligand binding studies suggest that activation of $5HT_2$ receptors is the primary mechanism of action of LSD and other hallucinogens (Rasmussen and Aghajanian, 1986; Heym and Jacobs, 1988; Cunningham and Appel, 1988; Titeler *et al.*, 1988). Furthermore, we have demonstrated that (+)LSD and another hallucinogen, 1-(2,5)-dimethoxy-4-methylphenyl-2-aminopropane, are partial agonists at $5HT_2$ receptors in rat cerebral cortex slices (Sanders-Bush *et al.*, 1988).

The amino acid sequence for the $5HT_2$ receptor suggests that it is a member of the family of G-protein-coupled neurotransmitter receptors (Pritchett *et al.*, 1988). Interestingly, the receptor shares 51% sequence homology with the $5HT_{1C}$ receptor (Lubbert *et al.*, 1987; Julius *et al.*, 1988). The pharmacological profiles of $5HT_2$ and $5HT_{1C}$ receptors are also very similar (Hoyer, 1988; Sanders-Bush *et al.*, 1990). In addition, both receptors are positively coupled to the hydrolysis of PI (Conn *et al.*, 1986). Given the striking similarities between $5HT_2$ and $5HT_{1C}$ receptors, we hypothesized that activation of $5HT_{1C}$ receptors might constitute an important action of hallucinogenic drugs. In accordance with this hypothesis, we have shown that the hallucinogens (+)LSD, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane, 1-(2,5-dimethoxy-4-bromphenyl)-2-aminopropane, 1-(2,5-dimethoxyphenyl-4-iodophenyl)-2-aminopropane, and 5-methoxy-N,N-dimethyltryptamine are agonists at $5HT_{1C}$ receptors (Sanders-Bush *et al.*, 1988; Sanders-Bush and Breeding, 1991). In the present study, we further characterize the interaction of (+)LSD with $5HT_{1C}$ receptors. In addition, the effects of two nonhallucinogenic congeners, lisuride and BOL, are compared at $5HT_{1C}$ and $5HT_2$ receptors.

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ABBREVIATIONS: LSD, lysergic acid diethylamide; 5HT, 5-hydroxytryptamine (serotonin); PI, phosphoinositide; IP, inositol-1-monophosphate; BOL, (+)-2-bromo-lysergic acid diethylamide.

Methods

Radioligand binding. Frozen brains from male Sprague-Dawley rats were obtained from Harlan Industries (Indianapolis, IN). Immediately after thawing the brains, choroid plexi were dissected and combined in 20 volumes of ice-cold 0.05 M Tris buffer, pH 7.6. The combined tissue was homogenized using a Brinkmann Polytron microprobe. The homogenate was centrifuged for 10 min at 11,000 × *g*. The resulting supernatant was carefully decanted and discarded. The pellet was washed twice more by resuspending it in fresh buffer followed by centrifugation. The final pellet was homogenized in buffer with a final tissue concentration of 10 mg/ml and stored at −70°C. On the day of an experiment, the sample was diluted with 0.05 M Tris buffer (pH 7.4), 0.1% ascorbic acid, 4 mM calcium chloride and 10 μM pargyline (final pH 7.4) to give a final tissue concentration of 1 mg/ml.

Membrane fractions were incubated at 37°C for 30 min with 1 nM ^{3}H-mesulergine and varying concentrations of competitor. Filtration was performed using a Brandel harvester with GF/C filters, previously soaked in 3% polyethylenimine, pH 7.0. Nonspecific binding was defined by adding 10 μM 5HT. Protein concentration was determined by the method of Bradford (1976), with bovine serum albumin as a standard.

Competition curves were constructed using 10 competing drug concentrations in duplicate. IC_{50} values were determined from Hill plots. Apparent K_d values were calculated by the method of Cheng and Prusoff (1973).

PI hydrolysis. Primary cultures of choroid plexus epithelial cells were prepared by a modification of the method of Crook *et al.* (1981). Choroid plexi from 20-day-old male Sprague-Dawley rats (Sasco, Inc., Omaha, NE) were rapidly dissected on ice and rinsed in Ca^{++}- and Mg^{++}-free Hanks' buffer containing type I DNase. The tissue was incubated for 10 min with 0.3 mg/ml pronase. The enzyme/buffer mixture was aspirated and the tissue gently agitated in fresh Hanks' buffer to liberate epithelial cells. The supernatant was collected, and the remaining sediment was washed twice with fresh Hanks' buffer. The pronase digestion was repeated twice. Pooled supernatants and washes were centrifuged for 5 min at 200 × *g* to spin down epithelial cells. The cell pellet was washed twice by resuspension in Hanks' buffer. The final pellet was resuspended in Hanks' buffer, and the cells were plated into 48-well culture dishes (11-mm diameter) containing 0.75 ml Ham's F-12 with 10% fetal bovine serum and 10 μg/ml gentamicin. Cells were maintained in a humidified incubator in an atmosphere of 5% CO_2 and 95% air at 37°C. On day 3 of culture, the medium was completely replaced with Ham's F-12 without serum. Cells were used in PI hydrolysis assays on days 5 to 7 of culture (2–4 days after serum removal).

NIH 3T3 fibroblasts transfected with $5HT_2$ receptor cDNA from rat brain (Julius *et al.*, 1990) were grown in 48-well culture dishes (11-mm diameter) containing 0.25 ml Dulbecco's modified Eagle medium with 10% bovine calf serum and 200 μg/ml of the neomycin analog, Geneticin [(G-418), Gibco Laboratories, Long Island, NY]. Cells were maintained in a humidified incubator in an atmosphere of 8% CO_2 and 92% air at 37°C. Cells were used in PI hydrolysis assays upon reaching confluency.

Agonist-induced PI hydrolysis was determined by a modification of the method of Berridge *et al.* (1982). Cultured cells were labeled by removing the medium and incubating for 18 hr with 0.25 ml of CMRL 1066 medium (Gibco Laboratories) containing 0.25 μCi of ^{3}H-myoinositol. Lithium chloride and pargyline were added to give final concentrations of 10 mM and 10 μM, respectively, and 15 min later agonist was added. The reaction was stopped after 30 min by aspirating the medium and adding a small volume (25 μl) of methanol to each well. The plates were air dried, 0.5 ml of buffer (20 mM NaCl, 5 mM Na_2EDTA and 10 mM Tris, pH 7.4) containing 2.5% trypsin was added to each well and, after standing for 10 min, the mixture was sonicated. A 0.3-ml aliquot was transferred to an assay tube, containing 0.9 ml chloroform/methanol (1:2). ^{3}H-IP was separated by anion exchange chromatography (Conn and Sanders-Bush, 1985).

In experiments involving fresh tissue, 20- to 30-day-old male Sprague-Dawley rats (Sasco, Inc.) were used. Choroid plexi in the lateral and third ventricles were removed by cutting through the dorsal roof of the lateral ventricles, grasping the exposed choroid plexus with fine forceps and pulling gently. ^{3}H-IP formation in intact choroid plexus or slices of cerebral cortex was performed according to the method of Conn *et al.* (1986).

Chemicals and drugs. Hanks' buffer, gentamicin, Geneticin (G-418), Ham's F-12 medium, fetal bovine serum, CMRL 1066 medium, Dulbecco's modified Eagle medium, and 48-well cell culture cluster dishes were obtained from Gibco Laboratories. Bovine calf serum was obtained from HyClone Laboratories Inc. (Logan, UT). Pronase was obtained from Boerhinger Mannheim (Indianapolis, IN). DNase (type 1), pargyline and 5HT creatinine sulfate were obtained from Sigma Chemical Co. (St. Louis, MO). Spiperone hydrochloride and mianserin hydrochloride were obtained from Research Biochemicals, Inc. (Natick, MA); atropine sulfate from Aldrich Chemical Co. (Milwaukee, WI); prazosin hydrochloride, from Pfizer Laboratories (New York, NY); and haloperidol from McNeil Laboratories (Fort Washington, PA). ^{3}H-myo-inositol (15.6 Ci/mmol) and Aquasol were purchased from DuPont, NEN Products (Boston, MA) and ^{3}H-mesulergine (70 Ci/mmol), from Amersham Corp. (Arlington Heights, IL). The following drugs were kindly donated by the indicated manufacturers: ketanserin tartrate by Janssen Pharmaceutica (Beerse, Belgium), triprolidine hydrochloride by Burroughs Wellcome Co. (Research Triangle Park, NC), mesulergine by Sandoz Research Institute (East Hanover, NJ) and lisuride hydrogen maleate by Berlex Laboratories (Cedar Knolls, NJ). (+)LSD tartrate, (−)LSD tartrate and BOL hydrogen tartrate were obtained from the National Institute of Drug Abuse (Rockville, MD).

Results

(+)LSD increased ^{3}H-IP formation in intact choroid plexus from the rat. This effect was concentration-dependent, with an EC_{50} value of 9 nM (fig. 1). Conversely, the nonhallucinogenic enantiomer, (−)LSD, did not stimulate PI hydrolysis in this tissue. Likewise, (+)LSD, but not the (−) isomer, increased ^{3}H-IP formation in cultures of rat choroid plexus epithelial cells

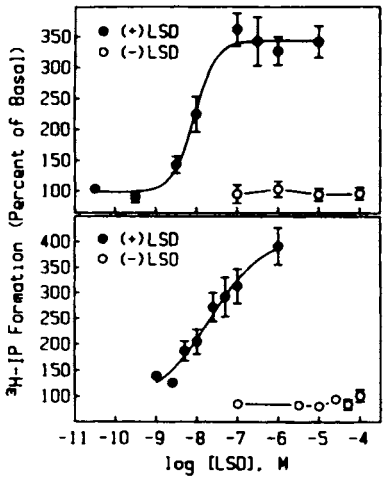

Fig. 1. Upper panel, stimulation of PI hydrolysis by the isomers of LSD in intact choroid plexus. ^{3}H-inositol-labeled tissue was incubated for 45 min with 10 mM LiCl, 10 μM pargyline and increasing concentrations of drug (n = 6). Radioactivity in the ^{3}H-IP fraction was expressed as a percentage of basal value and is plotted on the y-axis. The mean basal ^{3}H-IP formation was 3461 ± 332 cpm (n = 24). The vertical bars represent S.E.M. Lower panel, stimulation of PI hydrolysis by the isomers of LSD in primary cultures of choroid plexus epithelial cells. ^{3}H-inositol-labeled cells were incubated for 30 min with 10 mM LiCl, 10 μM pargyline and increasing concentrations of drug (n = 6–12). Radioactivity in the ^{3}H-IP fraction was expressed as a percentage of basal value and is plotted on the y-axis. The mean basal ^{3}H-IP formation was 82 ± 16 cpm (n = 9). The vertical bars represent S.E.M.

with an EC_{50} value of 26 nM (fig. 1). This effect was not species dependent, inasmuch as (+)LSD also stimulated ^{3}H-IP formation with an EC_{50} of approximately 2 nM in primary cultures of rabbit choroid plexus epithelial cells (data not shown). The effect of (+)LSD in both intact tissue and in cultured cells was inhibited by the $5HT_2/5HT_{1C}$ receptor antagonists ketanserin and mianserin, but spiperone, a more selective $5HT_2$ receptor antagonist, failed to block at an equivalent concentration (table 1). In addition, mesulergine, a ligand commonly used to radiolabel the $5HT_{1C}$ receptor (Pazos *et al.*, 1984), potently and completely blocked (+)LSD-stimulated PI hydrolysis (fig. 2). The effect of (+)LSD was, however, not blocked by antagonists of histaminergic, *alpha* adrenergic, or muscarinic cholinergic receptors (table 1).

BOL and lisuride, nonhallucinogenic congeners of LSD, potently blocked 5HT-stimulated PI hydrolysis in both intact choroid plexus and in cultured cells (fig. 3). Additionally, BOL and lisuride blocked (+)LSD-stimulated PI hydrolysis (fig. 4). Neither BOL nor lisuride increased PI hydrolysis when added alone (fig. 3). Both BOL and lisuride potently competed for ^{3}H-mesulergine binding to choroid plexus membranes, with apparent K_d values similar to the K_i values for inhibiting 5HT-stimulated PI hydrolysis (table 2). (−)LSD blocked 5HT-stimulated PI hydrolysis in intact choroid plexus with an IC_{50} of 160 μM (data not shown). (−)LSD also was a weak competitor for ^{3}H-mesulergine binding (K_i = 39 μM), which suggests that

TABLE 1

Antagonism of (+)LSD-stimulated PI hydrolysis in choroid plexus

Antagonists (1 μM) were added 15 min before the addition of 33 nM (+)LSD to choroid plexus epithelial cells in culture or 20 nM (+)LSD to intact choroid plexus. Mean basal ^{3}H-IP formation was 358 ± 53 cpm (n = 28) in cells and 2751 ± 219 cpm (n = 21) in tissue. Antagonists alone did not alter the basal values. The number of individual determinations is indicated in parentheses. ND, not determined.

	^{3}H-IP Formation	
	Cells	Intact
	% of Basal	
(+)LSD	301 ± 29 (21)	258 ± 19 (19)
Plus spiperone	277 ± 38 (16)	239 ± 23 (10)
Plus ketanserin	182 ± 17 (12)*	113 ± 14 (10)***
Plus mianserin	113 ± 22 (11)***	35 ± 2 (19)***
Plus atropine	ND	264 ± 19 (9)
Plus triprolidine	ND	256 ± 14 (9)
Plus prazosin	ND	260 ± 22 (8)

* $P < .05$, *** $P < .001$ compared with (+)LSD alone. Differences between mean values were determined by analysis of variance with a statistical program that calculates Bonferroni P values (Graph-Pad Instat, Intuitive Software for Science, San Diego, CA).

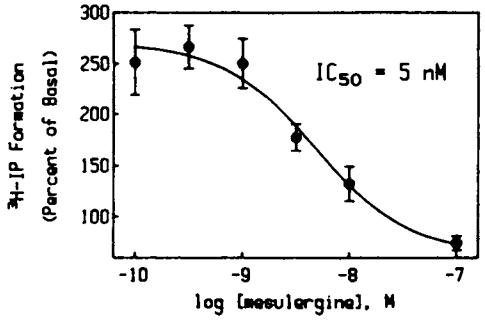

Fig. 2. Antagonism of (+)LSD-stimulated PI hydrolysis in intact choroid plexus by mesulergine. Conditions were the same as in figure 1. Mesulergine was added 15 min before the addition of 660 nM LSD (n = 8). The mean basal ^{3}H-IP formation was 2698 ± 195 cpm (n = 16). The vertical bars represent the S.E.M.

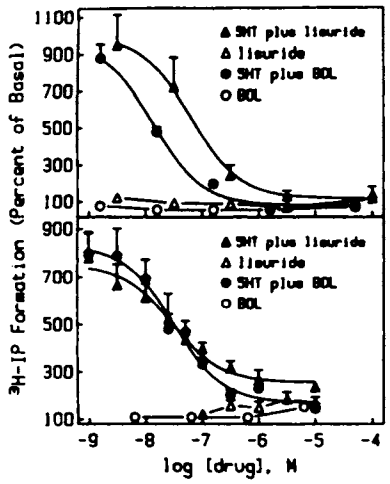

Fig. 3. Upper panel, antagonism of $5HT_{1C}$ receptor-mediated PI hydrolysis in intact choroid plexus by BOL and lisuride. Conditions were the same as in figure 1. BOL (n = 8) or lisuride (n = 6) was added 15 min before the addition of 100 nM 5HT. The mean basal ^{3}H-IP formation was 3253 ± 436 cpm (n = 20). The vertical bars represent S.E.M. Lower panel, antagonism of $5HT_{1C}$ receptor-mediated PI hydrolysis in primary cultures of choroid plexus epithelial cells by BOL and lisuride. Conditions were the same as in figure 1. BOL (n = 3–10) or lisuride (n = 4–11) was added 15 min before the addition of 5HT (50 nM). Mean basal ^{3}H-IP formation was 195 ± 21 cpm (n = 12). The vertical bars represent the S.E.M.

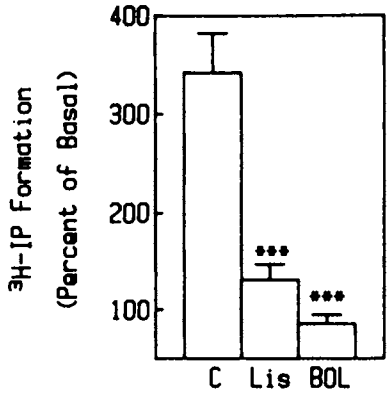

Fig. 4. Antagonism of (+)LSD-stimulated PI hydrolysis in intact choroid plexus by lisuride and BOL. Conditions were the same as in figure 1. Lisuride (3.3 μM) or BOL (3.3 μM) was added 15 min before the addition of 330 nM (+)LSD (n = 6). The mean basal ^{3}H-IP formation was 2499 ± 151 (n = 12). C, (+)LSD; Lis, (+)LSD plus lisuride; BOL, (+)LSD plus BOL. The vertical bars represent S.E.M. Differences between mean values were determined by analysis of variance. ***$P < 0.001$ compared with control (C).

TABLE 2

Antagonists of $5HT_{1C}$ receptor

IC_{50} values were determined from the concentration-response curves presented in figure 3 using a least square curve-fitting program (Graph-Pad Inplot, Intuitive Software for Science). K_i values were calculated by the method of Cheng and Prusoff (1973) with K_e values of 5HT determined previously (Sanders-Bush and Breeding, 1990, 1991). K_d values were determined in membranes from choroid plexus and are the mean ± S.E.M. for three determinations.

	PI Hydrolysis, K_i		Binding	
	Cells	Intact	K_d	Hill slope
	nM		nM	
BOL	18	4	5 ± 2	0.90
Lisuride	17	17	7 ± 1	0.85

this isomer has a much lower affinity for the $5HT_{1C}$ receptor than does the (+) isomer.

Slices of rat cerebral cortex have $5HT_2$ receptors coupled to PI hydrolysis (Conn and Sanders-Bush, 1985). Lisuride, but not BOL, produced a small increase in ^{3}H-IP formation in slices of rat cerebral cortex (fig. 5). The effects of 5HT, (+)LSD, BOL and lisuride were examined in cultures of NIH 3T3 cells transfected with rat brain $5HT_2$ receptor cDNA to explore further the interaction of these drugs with $5HT_2$ receptors. 5HT increased ^{3}H-IP formation in a concentration-dependent manner with an EC_{50} of 100 nM (fig. 6). (+)LSD potently increased ^{3}H-IP formation with an EC_{50} of 6 nM and a maximum effect that was 40% of that seen with 5HT (fig. 6). Lisuride also potently increased ^{3}H-IP formation with an EC_{50} of 30 nM (fig. 7). Consistent with the interpretation that lisuride is a partial agonist at the $5HT_2$ receptor, the effects of lisuride and 5HT were not additive. The effects of lisuride, as well as (+)LSD, were blocked by the $5HT_2$ receptor antagonists ketanserin, spiperone and mianserin, but not by antagonists of dopaminergic, *alpha* adrenergic or muscarinic cholinergic receptors (table 3). On the other hand, BOL completely blocked

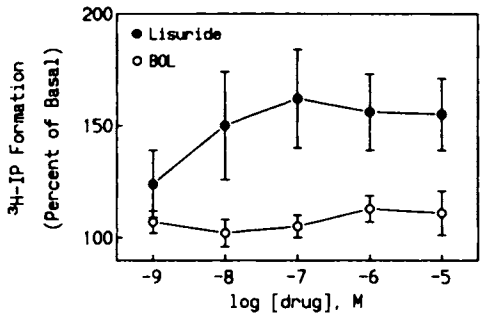

Fig. 5. Effects of nonhallucinogenic congeners of (+)LSD in rat cerebral cortex. ^{3}H-inositol-labeled slices were incubated for 30 min with 10 mM LiCl, 10 μM pargyline and increasing concentrations of drug. ^{3}H-IP formation was expressed as a percentage of basal value and is plotted on the *y*-axis. The data for BOL reflect the mean of five separate experiments, each done in triplicate. The mean basal ^{3}H-IP formation was 583 ± 109 cpm (n = 5). The maximum response to 5HT (3.3 μM) was 196 ± 25% of basal value (n = 5). The data for lisuride reflect the mean of six separate experiments, each done in triplicate. The maximum response to 5HT in these experiments was 309 ± 26% of basal value (n = 6). For statistical analyses, the effect of a maximum concentration of lisuride (1 μM) was compared with the basal response using a two-tailed Student's t test. The mean lisuride response was 563 ± 59 cpm (n = 6), which was significantly different ($P < .05$) from the basal value (361 ± 68 cpm, n = 6). The vertical bars represent the S.E.M.

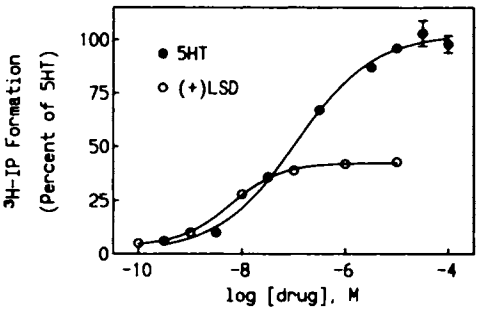

Fig. 6. Partial agonist effect of (+)LSD in cultures of fibroblasts transfected with $5HT_2$ receptor cDNA. ^{3}H-inositol-labeled cells were incubated for 30 min with 10 mM LiCl, 10 μM pargyline and increasing concentrations of drug. Radioactivity in the ^{3}H-IP fraction was expressed as a percentage of the maximum response to 5HT and is plotted on the *y*-axis. Vertical bars represent the S.E.M. (n = 9).

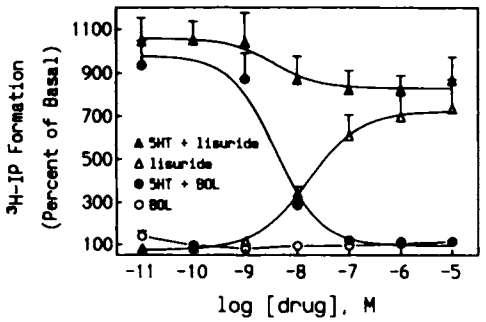

Fig. 7. Effect of lisuride and BOL on $5HT_2$ receptor-mediated PI hydrolysis in cultures of fibroblasts transfected with $5HT_2$ receptor cDNA. Cells were treated as in figure 6. Antagonists were added 15 min before stimulation with 100 nM 5HT. Radioactivity in the ^{3}H-IP fraction was expressed as a percentage of basal value and is plotted on the *y*-axis. The mean basal ^{3}H-IP formation was 257 ± 36 cpm (n = 12). The vertical bars represent S.E.M. (n = 6).

TABLE 3

Antagonism of (+)LSD and lisuride-stimulated PI hydrolysis in cells transfected with $5HT_2$ receptor cDNA

Antagonists (100 nM) were added 15 min before the addition of 33 nM (+)LSD or lisuride to NIH 3T3 fibroblasts transfected with $5HT_2$ receptor cDNA. Mean basal ^{3}H-IP formation was 172 ± 9 cpm (n = 24). The data reflect the mean ± S.E.M. of 12 determinations for (+)LSD or lisuride and six determinations for each antagonist.

	^{3}H-IP Formation	
	(+)LSD	Lisuride
	cpm	
Drug	1058 ± 80	756 ± 29
Plus spiperone	241 ± 27***	194 ± 9***
Plus ketanserin	237 ± 19***	182 ± 8***
Plus mianserin	364 ± 22***	243 ± 14***
Plus haloperidol	980 ± 66	677 ± 76
Plus prazosin	1236 ± 85	646 ± 34
Plus atropine	1091 ± 95	667 ± 42

*** $P < .001$ compared with (+)LSD or lisuride alone. Differences between mean values were determined by analysis of variance with a statistical program that calculates Bonferroni P values (Graph-Pad Instat, Intuitive Software for Science).

5HT-stimulated PI hydrolysis with no apparent agonist properties (fig. 7).

Discussion.

$5HT_{1C}$ receptors are present throughout the brain (Palacios and Dietl, 1988), but functional studies in brain are problematic because of the low density of sites. The choroid plexus, on the other hand, has a high density of $5HT_{1C}$ receptors (Pazos *et al.*, 1984; Yagaloff and Hartig, 1985) linked to the inositol lipid signaling pathway (Conn *et al.*, 1986). Choroid plexus epithelial cells can be cultured to greater than 90% homogeneity (Tsutsumi *et al.*, 1989). This system has been used as a model to characterize the action of hallucinogens at $5HT_{1C}$ receptors (Sanders-Bush and Breeding, 1991). A cell culture model localizes the effects of drugs to a particular cell type, minimizes possible indirect effects and allows better control of the receptor environment.

The present study confirms that (+)LSD potently activates PI hydrolysis in choroid plexus (Sanders-Bush *et al.*, 1988). (+)LSD is a potent agonist at $5HT_2$ receptors coupled to PI hydrolysis in rat cerebral cortex (Sanders-Bush *et al.*, 1988), and this has been confirmed by the use of fibroblasts transfected with the $5HT_2$ receptor cDNA. However, the effect of (+)LSD on PI hydrolysis in the choroid plexus is not mediated by activation of $5HT_2$ receptors, because there are presumably

no $5HT_2$ binding sites in this tissue (Pazos *et al.*, 1984; Yagaloff and Hartig, 1985; Hoyer *et al.*, 1986). Furthermore, antagonists block the (+)LSD response in choroid plexus with a rank-order of activity consistent with an interaction with $5HT_{1C}$, not $5HT_2$, receptors, *i.e.*, mesulergine > mianserin > ketanserin > spiperone.

BOL and lisuride, two nonhallucinogenic congeners of LSD, have been used as tools to examine the specificity of the effects of LSD. Actions shared by LSD and the nonhallucinogenic congeners are thought to be unrelated to the psychoactive properties of LSD, whereas actions of LSD that are not shared by BOL or lisuride are candidates for mediating hallucinogenic effects (for a review see White, 1986). In the present study, we have shown that although (+)LSD is a potent agonist, BOL and lisuride are potent antagonists of $5HT_{1C}$ receptors coupled to PI hydrolysis. Furthermore, BOL antagonizes the effect of LSD on PI hydrolysis, consistent with the clinical report that BOL attenuates the psychological effects of LSD in humans (Ginzel and Mayer-Gross, 1956).

For purposes of comparison, the effects of BOL and lisuride on $5HT_2$ receptor-mediated PI hydrolysis were examined. Slices of rat cerebral cortex have $5HT_2$ receptors coupled to the PI hydrolysis signaling cascade (Conn and Sanders-Bush, 1985). We have previously shown that (+)LSD is a partial agonist at these receptors (Sanders-Bush *et al.*, 1988). In the present study, BOL alone did not stimulate PI hydrolysis; however, lisuride did elicit a small PI hydrolysis response, perhaps by acting as a partial agonist at $5HT_2$ receptors. Studies of partial agonists using cerebral cortex are difficult because of the low $5HT_2$ receptor-mediated PI hydrolysis signal. NIH 3T3 cells transfected with $5HT_2$ receptor cDNA give a large 5HT response, which provides a sensitive model system for examining the effects of partial agonists. In this system, the partial agonist effects of (+)LSD and lisuride at $5HT_2$ receptors are clearly evident. Electrophysiological, behavioral and radioligand binding studies suggest that the hallucinogenic effects of (+)LSD are mediated by the activation of $5HT_2$ receptors. However, the present finding that $5HT_2$ receptor activation is a shared property of (+)LSD and the nonhallucinogen lisuride is not consistent with this hypothesis.

Although (+)LSD has been shown to bind and activate $5HT_{1C}$ receptors, it remains to be determined whether any part of its behavioral effects in man or animals is mediated by $5HT_{1C}$ receptors. A high correlation exists between the affinities of drugs at $5HT_{1C}$ receptors and their potencies to elicit a hallucinogenic discriminative cue in rats (Titeler *et al.*, 1988). However, this same series of compounds had a 10 to 100 times greater affinity for the $5HT_2$ receptor. The different affinities of hallucinogens for the two receptors may be explained by the selective labeling of the agonist affinity state of the $5HT_2$, but not the $5HT_{1C}$, site. Other evidence that $5HT_2$ receptors are involved in hallucinogen-induced behavior is based on reversal or prevention of these behavioral effects by $5HT_2$ receptor antagonists (for a review see Glennon, 1990). However, most antagonists of $5HT_2$ receptors also potently block $5HT_{1C}$ receptors (Sanders-Bush and Breeding, 1988). Spiperone is the most selective $5HT_2$ receptor antagonist currently available; it was compared with other $5HT_2$ receptor antagonists in animals trained to discriminate LSD from saline (Cunningham and Appel, 1987). In these studies, spiperone was unique in that it failed to block LSD discrimination, perhaps reflecting the involvement of $5HT_{1C}$ receptors in this behavior. Additional studies of the relative importance of $5HT_2$ *vs.* $5HT_{1C}$ receptors would be greatly facilitated by the development of new pharmacological tools that discriminate between the two receptor subtypes.

In conclusion, activation of $5HT_2$ receptors is currently believed to be the primary mechanism of action of LSD and other hallucinogens. However, few investigations have taken into account the $5HT_{1C}$ receptor. Given the evidence presented here that (+)LSD is an agonist at $5HT_{1C}$ receptors and nonhallucinogenic congeners are not, we propose that $5HT_{1C}$ receptor activation may be involved in the mechanism of action of (+)LSD.

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