0022-3565/96/2783-1373$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 278, No. 3

Printed in U.S.A.
JPET 278:1373–1382, 1996

LSD and the Phenethylamine Hallucinogen DOI are Potent Partial Agonists at 5-HT_{2A} Receptors on Interneurons in Rat Piriform Cortex[1,2]

GERARD J. MAREK and GEORGE K. AGHAJANIAN

Departments of Psychiatry (G.J.M., G.K.A.) and Pharmacology (G.K.A.), Yale University School of Medicine, Ribicoff Research Facilities of the Connecticut Mental Health Center, New Haven, Connecticut

Accepted for publication May 30, 1996

ABSTRACT

Correlations between 5-hydroxytryptamine (5-HT) receptor binding affinities and human hallucinogenic potency have suggested that 5-HT_2 receptors mediate the hallucinogenic effects of lysergic acid diethylamide (LSD) and phenethylamine hallucinogens. Electrophysiological studies have suggested that a subpopulation of γ-aminobutyric acid (GABA)ergic interneurons in layer III of the rat piriform cortex are excited by serotonin (5-HT) *via* 5-HT_{2A} receptors. These interneurons have inhibitory inputs on pyramidal cells in layer II. In the present study, we tested low concentrations of both LSD (3–100 nM) and the phenethylamine hallucinogen 1-(2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI; 0.3–10 μM) on rat piriform cortical interneurons that were excited by 5-HT. Both LSD (3–100 nM) and DOI (0.3–10 μM) excited almost every cell excited by 5-HT. The maximal excitation achieved with LSD and DOI was 39% and 55% of the effect of a near-maximal 5-HT concentration (100 μM). Consistent with a partial agonist action, LSD and DOI blocked the 5-HT excitation of piriform cortical interneurons only at the higher hallucinogen concentrations tested. A specific 5-HT_{2A} receptor antagonist, MDL 100,907, blocked excitation of these interneurons by 5-HT, LSD and DOI, but not by norepinephrine or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate. Again, consistent with a partial agonist action of the hallucinogens, intracellular experiments showed that a maximal concentration of DOI (10 μM) induced fewer postsynaptic inhibitory currents than did 5-HT (100 μM) in pyramidal neurons in layer II of the piriform cortex. Based on the present electrophysiological studies, we conclude that LSD and DOI, a phenethylamine hallucinogen, act as highly potent partial agonists at cortical 5-HT_{2A} receptors.

Correlations between 5-HT receptor binding affinity and the hallucinogenic potency of LSD and phenethylamine hallucinogens in humans have suggested that 5-HT_2 receptors mediate the hallucinogenic effects of LSD and phenethylamine hallucinogens (Glennon *et al.*, 1984; Titeler *et al.*, 1988). LSD has a similar affinity for the 5-HT_{2A} receptor (K_d: 0.5–2.5 nM) and for the 5-HT_{2C} receptor (K_d: 3.8–12 nM; Titeler *et al.*, 1988; Hoyer, 1988). The K_d of LSD for 5-HT_2 receptors is near the peak plasma levels measured after both i.v. and p.o. LSD administration (10–20 nM; Aghajanian and Bing, 1964; Hawks and Chiang, 1986). However, agreement does not exist regarding whether LSD and other hallucinogens act as full or partial agonists (Sanders-Bush *et al.*, 1988; Glennon, 1990; Sheldon and Aghajanian, 1990) or antagonists (Pierce and Peroutka, 1988, 1990) at cortical 5-HT_2 receptors.

Because the cerebral cortex has a higher density of 5-HT_{2A} receptors than most subcortical areas (Pazos *et al.*, 1985; Mengod *et al.*, 1990; Pompeiano *et al.*, 1994), and given the profound effects of LSD on sensory and cognitive processes, cortical 5-HT_{2A} receptors are likely to mediate many of the hallucinogenic actions of LSD. LSD (1 μM) was previously reported to act as an antagonist at 5-HT_2 receptors on a subpopulation of pyramidal neurons that are depolarized by 5-HT in the rat somatosensory cortex (Pierce and Peroutka, 1990). However, the depolarizations caused by 5-HT are only about 4 mV, making detection of partial agonist effects difficult, especially in light of the observation that LSD stimulates phosphoinositide hydrolysis in cerebral cortex to only 25% of the maximum response to 5-HT (Sanders-Bush *et al.*, 1988).

The rat piriform cortex has a population of GABAergic interneurons that are excited by 5-HT *via* 5-HT_{2A} receptors

Received for publication October 16, 1995.
[1] G.K.A. was supported by Public Health Service Grants MH17871 and MH14276 and the state of Connecticut.
[2] G.J.M. was supported by a Veterans Administration Schizophrenia Fellowship Grant.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; LSD, lysergic acid diethylamide; GABA, γ-aminobutyric acid; IPSPs, inhibitory postsynaptic potentials; DOI, 1-(2,5-dimethoxy-4-iodophenyl-2-aminoproprane; ACSF, artificial cerebrospinal fluid; NE, norepinephrine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; IPSCs, inhibitory postsynaptic currents.

(Sheldon and Aghajanian, 1990, 1991; Gellman and Aghajanian, 1993; Marek and Aghajanian, 1994). These GABAergic interneurons give rise to IPSPs in pyramidal neurons found in layer II of the piriform cortex (Sheldon and Aghajanian, 1990). These interneurons appear to represent an ideal model system for the study of 5-HT_{2A} receptors because the signal is robust and other 5-HT receptors do not appear to be present on these cells (Sheldon and Aghajanian, 1990, 1991). In contrast, cortical pyramidal cells often display both 5-HT_{2A} and 5-HT_{1A} responses (Davies *et al.*, 1987; Araneda and Andrade, 1991). Therefore, the present study was designed to investigate whether LSD and the phenethylamine hallucinogen DOI are agonists, partial agonists or antagonists at 5-HT_{2A} receptors on 5-HT-activated interneurons in piriform cortex.

Methods

Brain slice preparation. Brain slices were prepared from 60 male Sprague-Dawley rats (100–200 g) as described previously (Aghajanian and Rasmussen, 1989). Briefly, rats were anesthetized with chloral hydrate (400 mg/kg i.p.), in adherence to protocols approved by the Yale University Animal Care and Use Committee. After decapitation, the brain was removed rapidly and placed in an ice-cold modified ACSF in which sucrose (252 mM) was substituted for NaCl. A block of the piriform cortex was dissected free and coronal slices (500 μm) were cut with a vibrating-knife microtome (Vibraslice, WPI). A slice containing piriform cortex was then transferred to the stage of a fluid-gas interface chamber which had a constant flow of humidified 95% O_2-5% CO_2. The chamber was heated slowly from room temperature to 34°C. The slices were perfused with normal ACSF, which consisted of (in millimolar): NaCl, 126; KCl, 3; $CaCl_2$, 2; $MgSO_4$, 2; $NaHCO_3$, 26; NaH_2PO_4, 1.25 and D-glucose, 10. There was a 2-hr recovery period before beginning experiments.

Electrophysiological recordings. Extracellular recordings were conducted using an Axoclamp-2A (Axon Instruments, Burlingame, CA). Placement of the recording electrodes was facilitated by visualizing the three layers of the piriform cortex at low magnification under reflected light. Extracellular recordings from interneurons located in layer III of the piriform cortex were made using glass microelectrodes filled with 2 M NaCl (5–10 megohm) as described previously (Sheldon and Aghajanian, 1990). Cells were found by searching while the slice was perfused with 30 μM 5-HT in order to activate quiescent cells. Cells were identified as interneurons if they had short duration (0.1–0.5 msec) and low amplitude (0.25–1 mV) extracellular action potentials and the ability to sustain rapid firing rates as characterized previously by both extracellular and intracellular recordings (Sheldon and Aghajanian, 1990, 1991; Gellman and Aghajanian, 1993). Unlike the interneurons, pyramidal cells in layer II are not brought to threshold for firing by bath application of 5-HT (Sheldon and Aghajanian, 1990). Once an interneuron was located, the solution was switched from 30 μM 5-HT back to control ACSF and the 5-HT-activated cells gradually slowed and, in most cases, ceased firing. Then, the effects of 5-HT creatinine sulfate, (−)-NE bitartrate (Sigma Chemical Co., St. Louis, MO) and AMPA (RBI, Natick, MA) were tested. Concentration-response determinations for 5-HT alone were made by perfusing successively higher concentrations of 5-HT (10, 30 and 100 μM) for 2 min and then testing the next higher dose after the previous response returned to base line; the 10 μM 5-HT was near threshold and the 100 μM 5-HT was near maximal. To ensure equilibration, LSD and DOI always were applied for a 10-min period. When the effects of 5-HT, NE and AMPA were tested in cells treated with LSD or DOI, this was not performed until the response to LSD or DOI had clearly reached a maximum response. The response for each drug concentration was defined as the total number of counts during the 1-min period of maximal activation for a given cell. The 5-HT_{2A} receptor antagonist MDL 100,907 (Marion Merrell Dow Research Institute, Cincinnati, OH, kindly provided by Dr. C. Schmidt) was perfused for 15- to 30-min periods before and after hallucinogen treatment in different experiments.

All drugs were in ACSF, which was bath-applied by gravity feed and the solutions were switched by way of 3-way stopcocks (Baxter Healthcare, Valencia, CA). There is approximately a 40-sec "dead space" in this system from the time that each solution is turned on until the new solution reaches the slice.

Intracellular recording and single-electrode voltage clamping were conducted in layer II pyramidal cells using an Axoclamp 2 (Axon Instruments, Burlingame, CA). Stubby electrodes (≅8 mm, shank to tip) with relatively low capacitance and resistance (25–35 megohm) were pulled from filament-containing capillary tubing (1.5 mm) with a Brown-Flaming electrode puller (Sutter Instruments) and filled with 2 M KCl. Under voltage clamp, electrodes prepared in this manner had rapid settling times (50–75 μsec), allowing switching frequencies of 4 to 6 Hz and a loop gain of 10 nA/mV (30% duty cycle). Phase lag was used to prevent oscillations; false clamping was avoided by using optimal capacitance neutralization and by allowing settling to a horizontal base line, as verified by monitoring input voltage continuously. Pyramidal cells were identified according to criteria as previously described (Sheldon and Aghajanian, 1991). Only cells having a resting potential ≥65, an apparent input resistance of >25 megohm and a spike amplitude of >80 mV were used for recording. As previously noted (Sheldon and Aghajanian, 1990), most pyramidal cells in piriform cortex display spontaneous synaptic potentials with the characteristics of $GABA_A$-mediated IPSPs (*e.g.*, they are seen as depolarizing potentials with KCl-filled electrodes and are blocked by the $GABA_A$ antagonist bicuculline). In the present study, pyramidal cells were voltage-clamped at −90 mV to enhance the amplitude of the IPSCs, which were recorded. The voltage-clamp signals were low-pass filtered (1000 Hz) and data were acquired with a pCLAMP/Digidata 1200 system (Axon Instruments Inc.). IPSC frequencies were obtained from 10 successive episodes (1-sec duration) during the base line and drug treatment periods by means of Axograph software using a peak detect program.

Data analysis. The response for each drug was defined as the total number of spikes during the 1-min period of maximal activation for a given cell. The effects of 5-HT, NE and AMPA both before and after LSD, DOI or MDL 100,907 was tested using a paired *t* test.

Results

As reported previously, 5-HT excited a subpopulation of cells in layer III of the rat piriform cortex with the electrophysiological characteristics of interneurons (Sheldon and Aghajanian, 1990, 1991; Gellman and Aghajanian, 1993). Also as reported previously, 5-HT induced IPSCs in layer II pyramidal cells (Sheldon and Aghajanian, 1990, 1991; Gellman and Aghajanian, 1993, 1994). Many of the same interneurons that were excited by 5-HT were also excited by NE *via* stimulation of *alpha*-1B adrenoceptors (Sheldon and Aghajanian, 1988; Marek and Aghajanian, 1996). All interneurons tested were excited by the excitatory amino acid agonist AMPA. Fifty-five 5-HT-activated interneurons and five pyramidal cells were tested in the present study.

LSD and DOI excite piriform cortical interneurons. LSD (3–100 nM) induced a concentration-dependent increase in the firing rate of interneurons and the maximal firing rate produced by the highest concentration of LSD was 39 ± 7.7% of the maximal effect observed in the same neurons in response to 100 μM 5-HT (figs. 1 and 2). The lowest concentration of LSD (3 nM) applied for 10 min induced firing in only 2 of 4 cells tested that were activated by 5-HT. The two

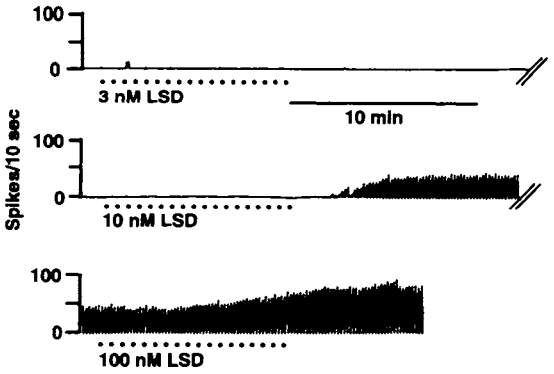

Fig. 1. Firing rate histogram of a quiescent piriform cortical interneuron during and after successive 10-min perfusions with 3, 10 and 100 nM LSD. Note that the excitation induced by 10 nM LSD continued through the 100 nM LSD perfusion and that the response to 100 nM LSD began 5 min after the beginning of the LSD application. Only two other cells were successively perfused with all three concentrations of LSD. This cell was used for illustrative purposes as all of the remaining cells were given a single concentration of LSD.

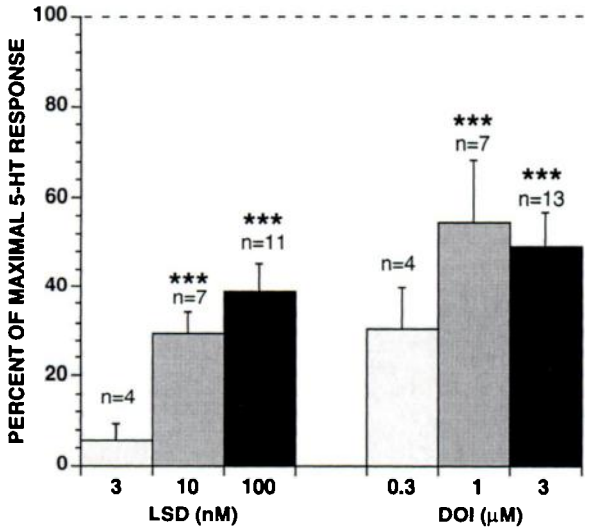

Fig. 2. Percent of maximal excitation of piriform cortical interneurons induced by LSD (3–100 nM) and DOI (0.3–3 μM) after a 10-min LSD or DOI perfusion compared to the maximal excitation induced by 100 μM 5-HT. Significantly different from the base line, ***P < .001.

interneurons that were excited by 3 nM LSD began to fire 25 min after the beginning of the LSD application and reached a plateau level of firing 7 to 14 min later. Figure 1 illustrates an interneuron that was activated by LSD in a dose-dependent manner; the interneuron was not induced to fire by 3 nM LSD but was excited by 10 and 100 nM LSD. The higher concentrations of LSD (10 or 100 nM) induced firing in 15 of 16 cells tested that were activated by 5-HT. The interneurons began to fire approximately 8 to 14 min after the beginning of the 10 and 100 nM LSD application and generally reached a plateau level of firing a few minutes later (figs. 1 and 3A). Note in figure 1 where the increment in firing induced by 100 nM LSD induced-firing began to occur 6 min after the beginning of the LSD application in a cell that continued to fire from a previous 10-min application of 10 nM LSD. In 12 of 14 cells this excitation lasted 20 min to more than 1 hr (figs. 1 and 4); in the remaining 2 cells this induction of firing was short-lived (fig. 3A). After 3 and 10 nM (fig. 3A) LSD, whether the response was short-lived or prolonged, the maximal response to 30 μM 5-HT was the same as it was to 5-HT before LSD treatment (table 1). However, the maximal response to 100 μM 5-HT was slightly but significantly reduced to 95 ± 1.5 and 85 ± 9.2% by 3 and 10 nM LSD. When the selective 5-HT$_{2A}$ receptor antagonist MDL 100,907 (30 nM) was applied for 15 min after the 10 nM LSD application, the response to 100 μM 5-HT, but not 100 μM NE, was blocked (n = 2, fig. 3B). Unlike the lower LSD concentrations, the highest LSD concentration (100 nM) almost totally prevented any additional effect by 100 μM 5-HT (table 1; figs. 4 and 5). In contrast, the maximal effect of 100 μM NE after even the higher concentration of LSD reached the same level as did the effect of 100 μM NE before LSD treatment (figs. 4 and 5). LSD (10 nM) increased the excitatory effect of AMPA to 202% of control (fig. 5). The increased excitability in response to AMPA after LSD was prolonged and seen even in the two cells with a short-lived response to LSD (fig. 3A). LSD (100 nM) also increased the excitatory effect of AMPA (to 344% of control; fig. 5).

DOI (0.3–3 μM, 10-min perfusion) induced firing in 23 of 24 cells tested that were also activated by 5-HT. DOI, 1 and 3 μM, excited piriform interneurons to 55 ± 14 and 49 ± 8%, respectively, of the firing rate observed in the same neurons to 100 μM 5-HT (fig. 2). The interneurons began to fire about 6 to 15 min after the beginning of the DOI perfusion and generally reached a plateau level of firing 12 to 20 min later (fig. 6). During and after this plateau period, the effect of 30 or 100 μM 5-HT brought cells to approximately the same firing rate as 5-HT before the 0.3 and 1.0 μM DOI treatments (fig. 7; table 1). A higher DOI concentration (3 μM) decreased the effect of 30 and 100 μM 5-HT to 62 ± 14 and 87 ± 6.5% of control, respectively (table 1; figs. 6 and 7). DOI (10 μM) decreased the effect of 100 μM 5-HT to 64 ± 12% of control (n = 3, P < .05, data not shown). Unlike 5-HT, the maximal effect of 100 μM NE after DOI (0.3–3.0 μM) reached the same level as did the effect of 100 μM NE before DOI treatment (figs. 6 and 7). All three concentrations of DOI potentiated the effect of 5 μM AMPA (figs. 6 and 7). DOI also potentiated the effect of 5 μM AMPA in the only interneuron in which DOI (0.3 μM) did not induce firing (data not shown).

Because LSD and DOI potentiated AMPA-induced excitation, experiments were conducted on piriform cortical interneurons to examine the interaction between 5-HT and AMPA. As with LSD and DOI, a low concentration of 5-HT (10 μM) had a greater than an additive effect with 5 μM AMPA (fig. 8A; table 2). The maximal firing rate after the combination of 5-HT (10 μM) and AMPA was 3-fold greater than the response to either 5-HT or AMPA alone. A high concentration of 5-HT (100 μM) had an additive effect with 5 μM AMPA (fig. 8B; table 2). As reported previously (Marek and Aghajanian, 1995), 5-HT did not have an additive effect with NE (table 2) as the maximal firing rate after the combined perfusion of 5-HT and NE was significantly less than the sum of 5-HT alone together with NE alone.

MDL 100,907 blocks LSD and DOI excitation of interneurons. To evaluate the effects of blockade of the 5-HT$_{2A}$ receptor upon the excitatory effects of LSD or DOI, we pretreated the slices with the selective 5-HT$_{2A}$ receptor antagonist MDL 100,907 (Palfreyman *et al.*, 1993) before application of LSD or DOI in 5-HT-activated interneurons. When MDL 100,907 (30 nM) was applied to the slice for 30 min before LSD (100 nM), only 1 of 4 cells was excited by LSD, in

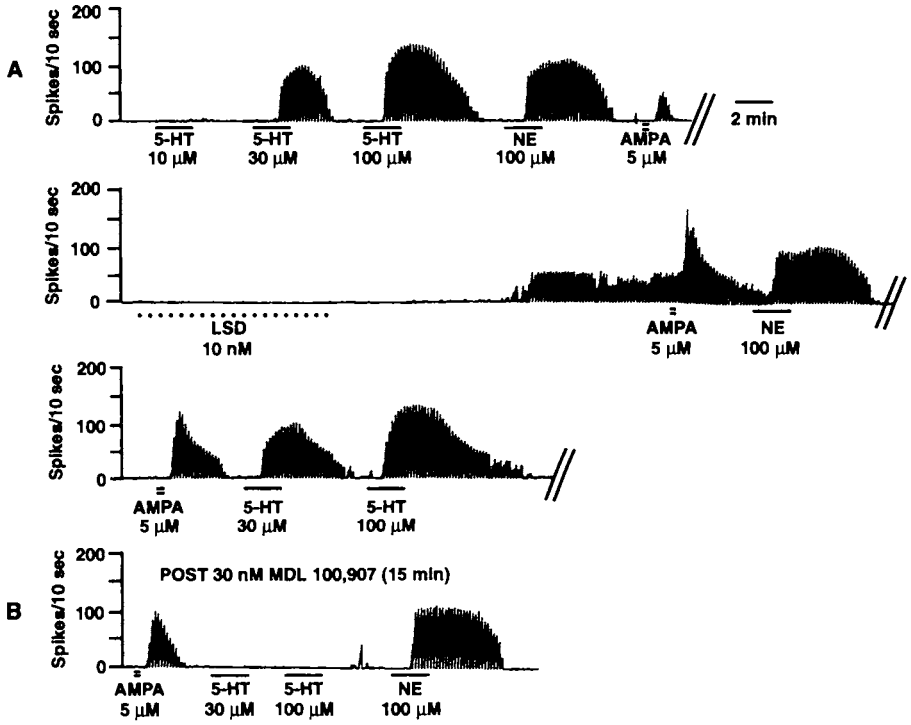

Fig. 3. Firing rate histogram of a piriform cortical interneuron before, during and after a 10-min perfusion with 10 nM LSD (A), and after a 15-min perfusion with 30 nM MDL 100,907 (B). 5-HT (10, 30 and 100 μM, indicated with solid lines), NE (100 μM, indicated with a solid line), AMPA (5 μM, indicated with a double line) and LSD (10 nM, indicated with a dotted line) were applied in ACSF for the times indicated.

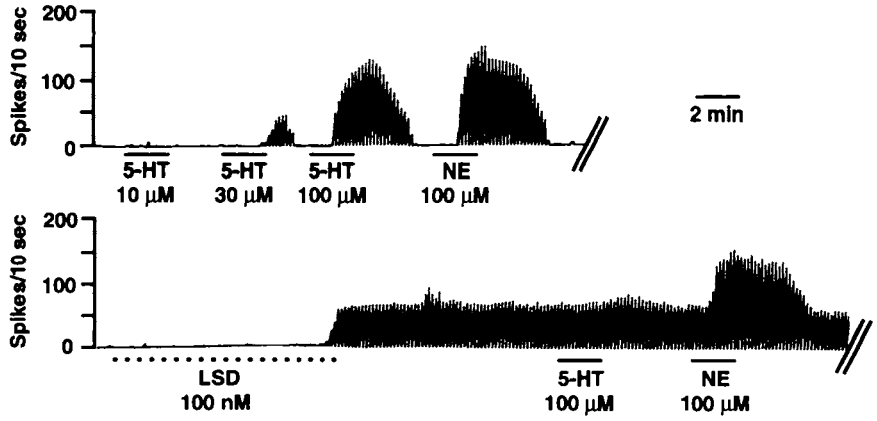

Fig. 4. Firing rate histogram of a piriform cortical interneuron before (top panel) and after (bottom panel) a 10-min perfusion with 100 nM LSD. 5-HT (10, 30 and 100 μM, indicated with solid lines), NE (100 μM, indicated with a solid line), and LSD (100 nM, indicated with a dotted line) were applied in ACSF for the times indicated. Note that the response of the interneuron to LSD began before the end of the 10-min LSD application.

contrast to 10 of 11 cells excited by LSD without MDL 100,907 pretreatment. MDL 100,907 decreased the firing rate in LSD-treated cells to 19% of control (fig. 9). Similarly, none of 4 cells tested were excited by DOI (3 μM) after a 15- to 30-min pretreatment with 30 nM MDL 100,907. In contrast all cells tested (n = 13) without MDL 100,907 pretreatment were excited by 3 μM DOI. Thus, MDL 100,907 decreased the firing rate in DOI-treated cells to 0% of control (fig. 9). In these experiments, MDL 100,907 also blocked the induction of firing in response to 100 μM 5-HT by 84%, but did not block the effects of 100 μM NE (n = 5) or 5 μM AMPA (n = 7, fig. 9).

DOI induces IPSCs in piriform cortical pyramidal neurons. DOI was tested for its ability to induce IPSCs in piriform cortical pyramidal neurons because 5-HT has been shown to induce IPSPs in piriform cortical pyramidal neurons by exciting GABAergic interneurons in a tetrodotoxin-sensitive manner (Sheldon and Aghajanian, 1990, 1991; Gellman and Aghajanian, 1993). Because preliminary experiments with 10 μM DOI found that this concentration excited GABAergic interneurons to the same degree observed with 1 and 3 μM DOI, we tested only a single high concentration of DOI (10 μM) for technical reasons to minimize the latency of the response to DOI so as not to prolong unnecessarily the experiments being performed under conditions of voltage-clamp. As noted previously (Sheldon and Aghajanian, 1990; Gellman and Aghajanian, 1993, 1994) for IPSPs, spontaneous reverse IPSCs are observed when using 2 mM KCl-filled electrodes (fig. 10, A and E). DOI was tested in 5 separate cells where 5-HT (100 μM) clearly induced an increase in IPSCs. DOI (10 μM) induced IPSCs (fig. 5) in all 5 of these cells with a latency of 6 to 8 min. After subtracting the frequency of basal IPSCs from both the 5-HT- and DOI-induced IPSCs, the frequency of IPSCs induced by DOI was 49 (±14)% of that induced by 100 μM 5-HT (fig. 10). This percentage is similar to that observed in comparing the direct effects of DOI and 5-HT on the firing of interneurons (see above). It should be noted that the frequency of IPSCs in

TABLE 1

Peak firing rate (Hz) of piriform cortical interneurons to agonist (5-HT, NE and AMPA) before and after hallucinogens[a,b]

Treatment	30 μM 5-HT	100 μM 5-HT
Agonist	14.0 ± 1.1	20.4 ± 0.3
LSD (3 nM)	0.5 ± 0.3	0.6 ± 0.3
Agonist + LSD[c]	13.2 ± 1.0	19.3 ± 0.3**
	n = 3	*n* = 3
Agonist	13.0 ± 1.0	19.6 ± 1.4
LSD (10 nM)	4.1 ± 0.9	4.4 ± 1.1
Agonist + LSD	11.9 ± 1.1	16.8 ± 1.8*
	n = 7	*n* = 7
Agonist	12.4 ± 1.7	16.8 ± 1.4
LSD (100 nM)	5.5 ± 1.4	5.0 ± 1.0
Agonist + LSD	6.6 ± 1.8*	7.7 ± 1.3**
	n = 5	*n* = 9
Agonist	10.1 ± 1.4	12.7 ± 1.2
DOI (0.3 μM)	2.5 ± 1.3	2.4 ± 1.3
Agonist + DOI	10.2 ± 1.6	12.7 ± 1.4
	n = 4	*n* = 4
Agonist	10.5 ± 0.8	15.4 ± 1.6
DOI (1.0 μM)	5.2 ± 0.2	7.5 ± 1.1
Agonist + DOI	8.5 ± 1.3	14.6 ± 1.5
	n = 2	*n* = 7
Agonist	12.4 ± 0.9	15.2 ± 0.8
DOI (3.0 μM)	5.0 ± 1.3	4.9 ± 1.3
Agonist + DOI	7.7 ± 1.7**	13.1 ± 1.0***
	n = 11	*n* = 10

[a] Firing rate (Hz) represents mean ± SE.
[b] 5-HT was tested after LSD or DOI only after a maximum response to the hallucinogen had clearly occurred in each cell.
[c] Agonist + LSD/DOI refers to total agonist effect after LSD/DOI.
* $P < .05$; ** $P < .01$ and *** $P < .001$, compared to pre-LSD or DOI response.

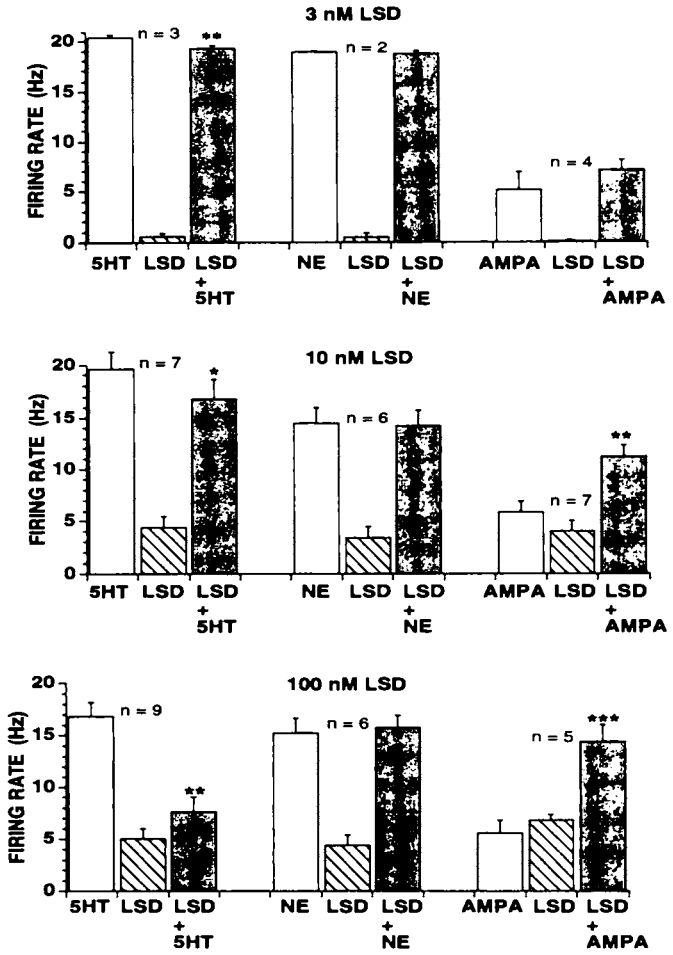

Fig. 5. Maximal response to 5-HT (100 μM), NE (100 μM) and AMPA (5 μM) both before and after 10-min perfusion with 3 nM (top panel), 10 nM (middle panel) or 100 nM (bottom panel) LSD. The open bars present the effects of 100 μM 5-HT, 100 μM NE or 5 μM AMPA before LSD application. The hatched bars present the effects of LSD on the firing rate for the min period immediately before retesting with 5-HT, NE or AMPA. The shaded bars present the maximal response to 5-HT, NE or AMPA when tested again after the LSD perfusion and thus present the combined effects of LSD and 5-HT, NE or AMPA. 5-HT, NE and AMPA were retested in these cells only after LSD had clearly reached a maximum response. *$P < .05$; **$P < .01$; ***$P < .001$.

pyramidal cells is only an approximation to the firing rate frequencies of 5-HT excited interneurons because: 1) there are spontaneously active GABAergic interneurons that are inhibited by 5-HT through 5-HT$_{1A}$ receptor (Sheldon and Aghajanian, 1990) and 2) DOI will affect only interneurons excited by 5-HT$_2$ receptor stimulation, whereas 5-HT can inhibit as well as excite different interneurons. Nevertheless, the present results show that in pyramidal cells DOI induces one-half as many IPSCs compared to 5-HT, just as DOI excited interneurons to one-half the firing rate compared to 5-HT.

Discussion

These studies were designed to evaluate whether the hallucinogenic drugs LSD and DOI are agonists, partial agonists or antagonists at 5-HT$_{2A}$ receptors using the excitation of piriform cortical interneurons by 5-HT as a model system. In general, the results show that both LSD and DOI are agonists rather than antagonists in this system because both hallucinogens excited virtually all piriform cortical interneurons that were excited by 5-HT. Furthermore, the results suggest that LSD and DOI are partial agonists at cortical 5-HT$_{2A}$ receptors because the maximal excitation produced by LSD and DOI was only 40 to 55% of the excitation induced by a near-maximal 5-HT concentration. Consistent with a partial agonist action, only high concentrations of LSD and DOI substantially reduced the maximal excitation induced by 5-HT. The blockade of LSD, DOI and 5-HT by MDL 100,907, a potent and specific 5-HT$_{2A}$ antagonist (Sorensen *et al.*, 1993; Marek and Aghajanian, 1994), indicates that both LSD and DOI, as well as 5-HT, are acting through 5-HT$_{2A}$ receptors. The present results provide the first electrophysiological demonstration that cortical 5-HT$_{2A}$ receptors are stimulated potently by LSD. In contrast to a previous suggestion for LSD (Pierce and Peroutka, 1988, 1990), these results show that LSD is not a 5-HT$_{2A}$ receptor antagonist in the cerebral cortex. Instead, the present results demonstrate that both LSD and DOI are potent partial agonists at piriform cortical 5-HT$_{2A}$ receptors, in agreement with previous work suggesting that LSD (Sanders-Bush *et al.*, 1988) and DOI (Pierce and Peroutka, 1988) are partial agonists at stimulating frontal cortical 5-HT$_{2A}$ receptor-mediated PI hydrolysis.

The onset of the excitation of interneurons by both LSD and DOI was slow compared to both 30 and 100 μM 5-HT. This slow onset could be an artefact of extracellular recording in that a response is not seen until the cell is brought to threshold for firing. However, intracellular recordings in piri-

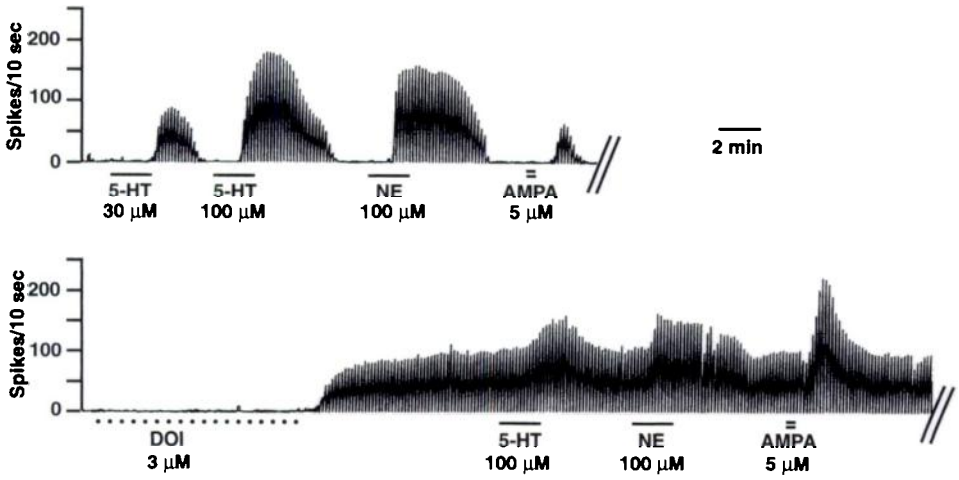

Fig. 6. Firing rate histogram of a piriform cortical interneuron before (top panel) and after (bottom panel) a 10-min perfusion with 3 μM DOI. 5-HT (30 and 100 μM, indicated with solid lines), NE (100 μM, indicated with solid line), AMPA (5 μM, indicated with a double line) and DOI (3 μM, indicated with a dotted line) were applied in ACSF for the times indicated.

form cortical interneurons with a maximal concentration of DOI demonstrated that the time to both onset and plateau for the response was over 4 times the corresponding times for the 100 μM 5-HT concentration (unpublished observations). Similar delayed responses for LSD and DOI were previously observed in the facial motor nucleus (Garratt *et al.*, 1993). The slow onset of the effects of hallucinogens might be related to the slow rate of association with the 5-HT_{2A} receptor for both LSD (Bennett and Snyder, 1975; Kadan *et al.*, 1984) and the phenethylamines such as DOI (Lyon *et al.*, 1987). Bennett and Snyder (1975) reported that binding of LSD to 5-HT_{2A} receptors at 37°C did not reach equilibrium until about 6 min; the rate of binding was even slower at lower temperatures. A slow rate of association of LSD for the platelet 5-HT_{2A} receptor has also been observed (Geaney *et al.*, 1984). Functionally, 5-HT induces a maximal shape change in platelets with a latency less than 1 min, whereas the maximal shape change induced by LSD in platelets does not occur until 5 to 10 min (Laubscher *et al.*, 1981). These observations are of interest to the present findings because the 5-HT-induced platelet shape change is mediated by 5-HT_{2A} receptors (Geaney *et al.*, 1984) and the slow onset of the LSD response occurs even in the absence of any difficulties with penetration through the tissue. Also in agreement with the above work, Burris and Sanders-Bush (1992) observed slow dissociation of LSD from the 5-HT_{2A} receptor and suggested that this was the reason why LSD, in addition to a partial agonist effect, blocked 5-HT_{2A} receptors in an unsurmountable manner using a cell line transfected with brain 5-HT_{2A} receptors.

Other factors might also play a role in the slow onset of the effects of the hallucinogens. Because the hallucinogens are partial agonists at the 5-HT_{2A} receptor, a higher percentage occupancy of receptors may be needed for a given response. Interestingly, the slow onset of the hallucinogens in slice preparations is paralleled by the observation that humans do not report subjective effects of i.v. LSD until about 5 min after the i.v. bolus (unpublished observations from Aghajanian and Bing, 1964). It remains to be determined to what degree the slow onset of the hallucinogens is related to a lower efficacy in stimulating the 5-HT_{2A} receptor as compared to the factor of hallucinogen binding kinetics.

A nonadditive interaction occurred between the hallucinogens and NE, which differed in some important respects from

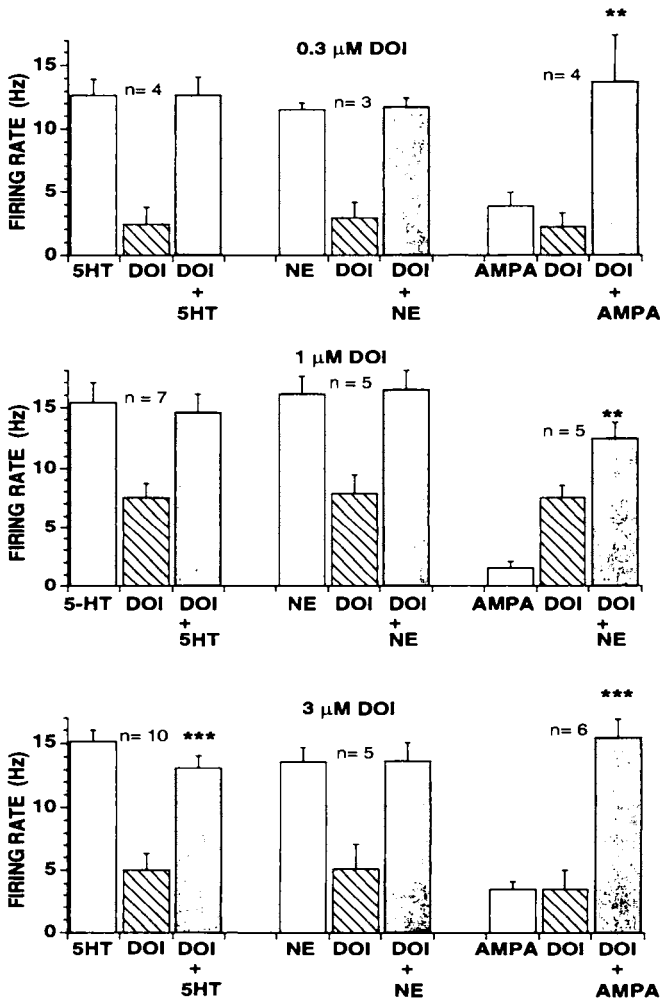

Fig. 7. Maximal response to 5-HT (100 μM), NE (100 μM) and AMPA (5 μM) both before and after 10-min perfusion with 0.3 μM (top panel), 1 μM (middle panel) or 3 μM DOI (bottom panel). The open bars present the effects of 100 μM 5-HT, 100 μM NE or 5 μM AMPA before DOI application. The hatched bars present the effects of DOI on the firing rate for the min period immediately before retesting with 5-HT, NE or AMPA. The shaded bars present the maximal response to 5-HT, NE or AMPA when tested again after the DOI perfusion and thus present the combined effects of DOI and 5-HT, NE or AMPA. 5-HT, NE and AMPA were retested in these cells only after DOI had clearly reached a maximum response. **P < .01; ***P < .001.

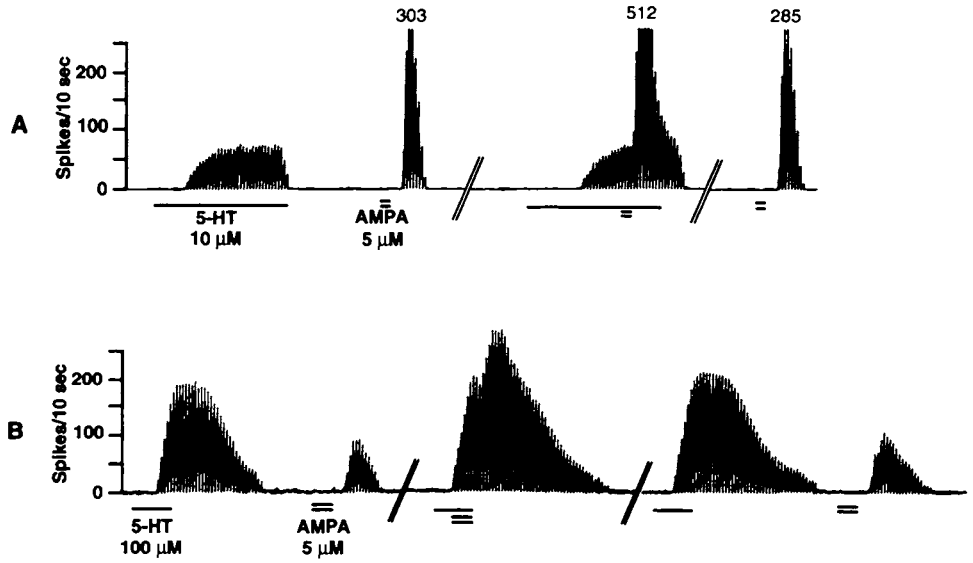

Fig. 8. Firing rate histograms of two separate piriform cortical interneurons given either a low concentration of 5-HT (10 μM; A) or a high concentration of 5-HT (100 μM; B) both alone and in combination with 5 μM AMPA. 5-HT (10 or 100 μM, indicated with solid lines) and AMPA (5 μM, indicated with a double line) were applied in ACSF for the times indicated. The number displayed above the AMPA responses in (A) correspond to the peak spikes/10 sec as the response went off the chart recorder scale.

TABLE 2
AMPA and NE interactions with 10 μM or 100 μM 5-HT

Drug	*n*	Firing Rate (Hz)[a]
5-HT (10 μM)	7	7.0 ± 1.4
AMPA (5 μM)	7	7.1 ± 1.3
5-HT (10 μM) and AMPA	7	21.6 ± 3.2[b,c]
5-HT (100 μM)	6	18.9 ± 1.5
AMPA (5 μM)	6	8.4 ± 2.8
NE (100 μM)	6	14.0 ± 2.0
5-HT (100 μM) and AMPA	6	25.0 ± 2.6[b]
5-HT (100 μM) and NE	6	20.1 ± 1.9[d]

[a] Firing rate (Hz) represents mean ± S.E.
[b] $P < .01$, compared to 5-HT alone.
[c] $P < .01$, compared to the sum of 5-HT alone and AMPA alone.
[d] $P < .001$, compared to the sum of 5-HT alone and NE alone.

the interaction between the hallucinogens and 5-HT. In comparing 5-HT and NE responses in these cortical interneurons, it is important to note that 100 μM concentrations of 5-HT and NE result in maximal responses (Marek and Aghajanian, 1994, 1996). The maximal response to the combination of a hallucinogen and NE was approximately the same as NE alone. Previous work (Marek and Aghajanian, 1995) and present observations suggest that 5-HT and NE share a common postreceptor transduction mechanism in exciting cortical interneurons. Specifically, an additive peak amplitude was not obtained for co-application of 5-HT (100 μM) and NE (100 μM) as would be expected for 2 receptors (5-HT$_{2A}$ and *alpha*-1B adrenergic receptors) that are coupled to the same postreceptor transduction system. The additive peak amplitude of 5-HT and AMPA show that the lack of an additive peak amplitude for the combination of 5-HT and NE is not due to a ceiling effect. Similarly, the present results indicate that LSD and DOI also share a common postreceptor transduction mechanism with NE because the peak amplitude of NE together with the amplitude of LSD and DOI is not additive. The interaction of 5-HT with the hallucinogens was also nonadditive, but differed from the NE-hallucinogen interaction in that the hallucinogens actually caused a concentration-dependent reduction in the maximal excitation seen after 5-HT. These results are consistent with occupancy of the receptor by LSD and DOI as partial agonists in the case of 5-HT, but not NE. The failure to show additivity in

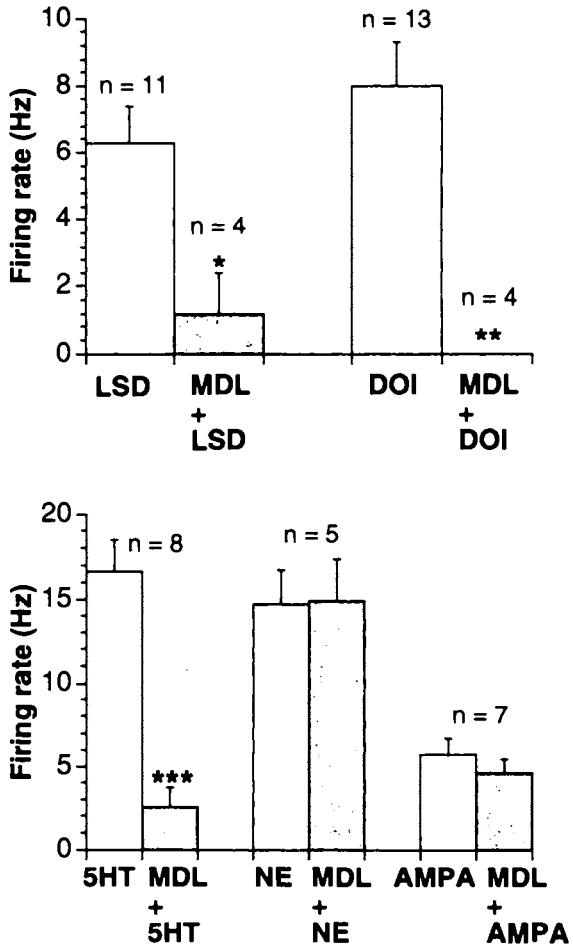

Fig. 9. The blockade by MDL 100,907 of the LSD- or DOI-induced excitation of piriform cortical interneurons (top panel) and the specificity of MDL 100,907 at blocking 5-HT with respect to NE and AMPA. The maximal firing rate of interneurons perfused for 10 min with LSD (100 nM) or DOI (3 μM) was compared to the maximal firing of a separate group of interneurons perfused for 10 min with LSD or DOI after a 15- to 30-min pretreatment with MDL 100,907 (30 nM; top panel). Before testing of these cells with LSD or DOI, the effects of MDL 100,907 on the response of these interneurons to 100 μM 5-HT, 100 μM NE and 5 μM AMPA was compared to the response to the agonists before the MDL 100,907 treatment (bottom panel). *$P < .05$; **$P < .01$; ***$P < .001$.

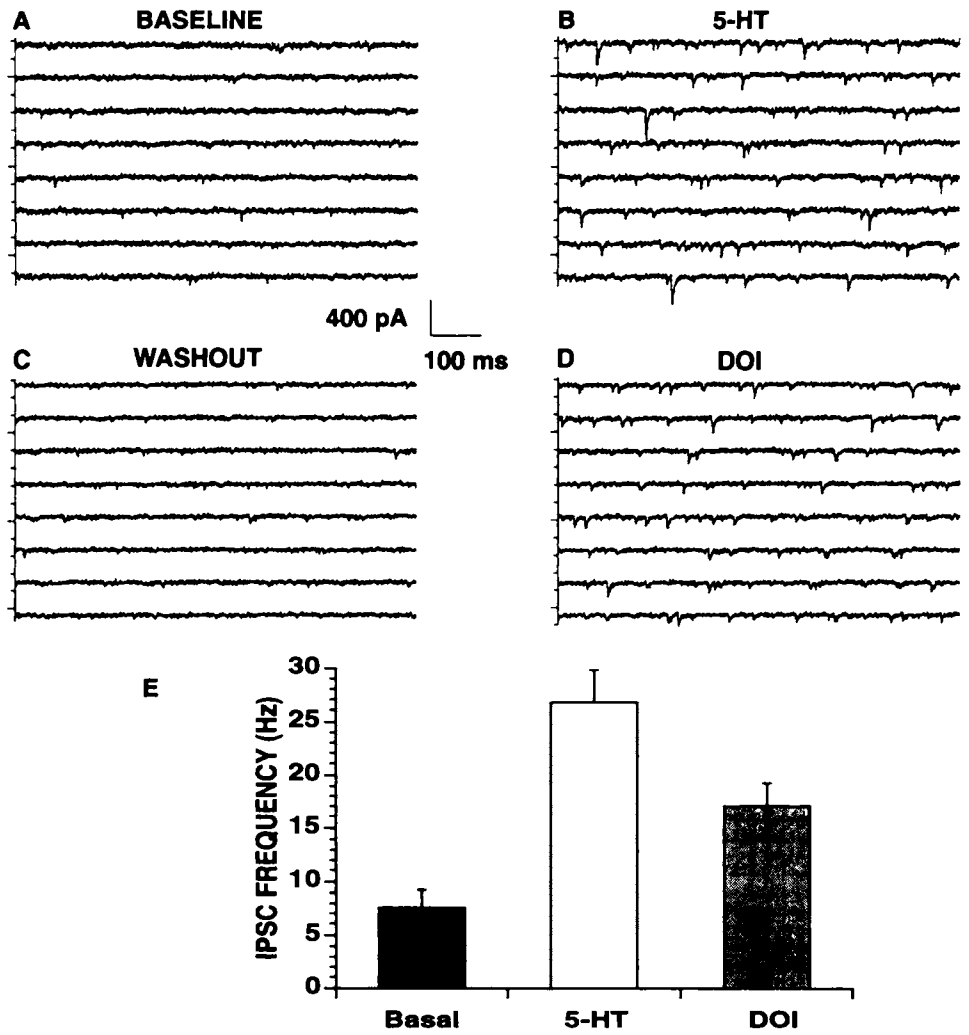

Fig. 10. Inhibitory postsynaptic currents in a piriform pyramidal neuron recorded under voltage-clamp conditions both before 5-HT (base line; A), during 5-HT (100 μM; B), after 5-HT washout (C) and during DOI (10 μM; D). For each condition, a set of eight 1-sec sweeps are shown. Note that a few relatively high-amplitude IPSCs that were induced by 5-HT were not seen after DOI. Also shown is a summary of the experiments (E) with the frequencies of IPSCs observed under basal conditions, 100 μM 5-HT and 10 μM DOI.

these experiments was not due to a ceiling effect because the excitation induced by AMPA, an ionotropic glutamate agonist, was potentiated by both LSD and DOI.

The present results are in agreement with previous suggestions that 5-HT_2 receptors (Glennon *et al.*, 1984; Rasmussen and Aghajanian, 1986; Garratt *et al.*, 1993) represent a common target of action for both indoleamine and phenethylamine hallucinogens. In particular, the observation that an indoleamine, LSD, and a phenethylamine, DOI, have similar electrophysiological effects at cortical 5-HT_{2A} receptors is consistent with the proposal that the 5-HT_{2A} receptor mediates the hallucinogenic effects of both indoleamine and phenethylamine hallucinogens (Titeler *et al.*, 1988). A recent report (Schreiber *et al.*, 1994) that a series of selective 5-HT_{2A} receptor antagonists, but not a 5-$HT_{2B/2C}$ antagonist, block the discriminative stimulus effects of the phenethylamine DOI also supports the hypothesis that both indoleamine and phenethylamine hallucinogens have behaviorally significant agonist effects at the 5-HT_{2A} receptor. However, a role for the 5-HT_{2C} receptor cannot be ruled out (Sanders-Bush and Breeding, 1991).

Other 5-HT receptors do not appear to be likely candidates for mediating the hallucinogens' effects of both indoleamine and phenethylamine hallucinogens. Although LSD potently binds to 5-$HT_{1A/1B/1D/1E/1F}$ receptors (Hoyer, 1988; Lovenberg *et al.*, 1993a), the phenethylamine hallucinogens do not (Titeler *et al.*, 1988; Pierce and Peroutka, 1989; Zgombick *et al.*, 1992; Adham *et al.*, 1993). LSD does not bind potently to either the 5-HT_3 or the 5-HT_4 receptor (Peroutka and Hamik, 1988; Gerald *et al.*, 1995). Inasmuch as LSD has relatively high affinity for the 5-$HT_{5A/5B/6/7}$ receptors (Matthes *et al.*, 1993; Monsma *et al.*, 1993; Ruat *et al.*, 1993), phenethylamine hallucinogens do not bind potently or act as an agonist at the 5-HT_5 or the 5-HT_7 receptor (Erlander *et al.*, 1993; Lovenberg *et al.*, 1993b). Thus, the 5-HT_{2A} and 5-HT_{2C} receptors remain as the most likely shared targets of action for both indoleamine and phenethylamine hallucinogens. Given the predominance of 5-HT_{2A} receptors over 5-HT_{2C} receptors in most areas of the neocortex (Pompeiano *et al.*, 1994; Wright *et al.*, 1995), the 5-HT_{2A} receptor appears to be the most likely candidate to mediate the major hallucinogenic effects of indoleamine and phenethylamine hallucinogens.

The present studies are in agreement with behavioral studies reviewed elsewhere (Glennon, 1990) which argue that the effects of LSD may be mediated by at least partial agonist rather than antagonist properties at the 5-HT_{2A} receptor. The present studies also are consistent with evidence in a cloned pituitary cell line that DOI and LSD decrease the density of 5-HT_{2A} receptors as a consequence of their potent partial agonist properties (Ferry *et al.*, 1993). One striking

feature of the present study is that the EC_{50} for LSD at inducing firing in piriform cortical interneurons is between 3 and 10 nM, whereas the binding affinity of LSD for the 5-HT$_{2A}$ receptor is between 0.5 to 10 nM (Bennett and Snyder, 1975; Peroutka and Snyder, 1979; Kadan *et al.*, 1984; Titeler *et al.*, 1988; Hoyer, 1988). Another striking feature of the present study is the observation that the agonist effects of LSD and DOI predominate over the antagonist effects until concentrations of 100 nM and 10 μM, respectively, are reached. In practice, such high concentrations normally would not be reached because, for example, the plasma levels of LSD after a 2 μg/kg i.v. bolus infusion in human subjects were about 10 to 20 nM for the first 4 hr after the LSD administration (Aghajanian and Bing, 1964). Similarly, the peak plasma concentration after p.o. administration of a 140-μg LSD dose was about 15 nM (Hawks and Chiang, 1986). Thus, the present *in vitro* data on cortical 5-HT$_{2A}$ receptors suggest that the hallucinogenic effects of LSD are due to an agonist, rather than an antagonist, action at 5-HT$_{2A}$ receptors.

There is currently a resurgence of LSD use. However, there is no established effective treatment for the acute adverse effects of this hallucinogen. Selective 5-HT$_{2A}$ antagonists, but not 5-HT$_{2C}$ antagonists, have been shown to block the discriminative stimulus effects of DOI (Schreiber *et al.*, 1994). Based on the present results, potent 5-HT$_{2A}$ receptor antagonists such as MDL 100,907 and risperidone (Gellman and Aghajanian, 1994) may block the untoward acute effects of LSD and other indoleamine and phenethylamine hallucinogens. The relatively low potency of phenothiazines and butyrophenones in blocking 5-HT$_{2A}$ receptors in relation to their dopamine receptor affinities (Rasmussen and Aghajanian, 1988) may account for their limited usefulness in treating acute adverse effects of LSD use.

Acknowledgments

We thank Nancy Margiotta for excellent technical assistance and Leslie Fields for secretarial assistance.

References

Adham, N., Kao, H.-T., Schechter, L. E., Bard, J., Olsen, M., Urquhart, D., Durkin, M., Hartig, P. R., Weinshank, R. L. and Branchek, T. A.: Cloning of another serotonin receptor (5-HT$_{1F}$): A fifth 5-HT$_1$ receptor subtype coupled to the inhibition of adenylate cyclase. Proc. Natl. Acad. Sci. U.S.A. **90:** 408–412, 1993.

Aghajanian, G. K. and Bing, O. H. L.: Persistance of lysergic acid diethylamide in the plasma of human subjects. Clin. Pharmacol. Ther. **5:** 611–614, 1964.

Aghajanian, G. K. and Rasmussen, K.: Intracellular studies in the facial nucleus illustrating a simple new method for obtaining viable motoneurons in adult rat brain slices. Synapse **3:** 331–338, 1989.

Araneda, R. and Andrade, R.: 5-hydroxytryptamine$_2$ and 5-hydroxytryptamine$_{1A}$ receptors mediate opposing responses on membrane excitability in rat association cortex. Neuroscience **40:** 399–412, 1991.

Bennett, J. P. and Snyder, S. H.: Stereospecific binding of D-lysergic acid diethylamide (LSD) to brain membranes: Relationship to serotonin receptors. Brain Res. **94:** 523–544, 1975.

Burris, K. D. and Sanders-Bush, E.: Unsurmontable antagonism of brain 5-hydroxytryptamine$_2$ receptors by (+)-lysergic acid diethylamide and bromo-lysergic acid diethylamide. Mol. Pharmacol. **42:** 826–830, 1992.

Davies, M. F., Deisz, R. A., Prince, D. A. and Peroutka, S. J.: Two distinct effects of 5-hydroxytryptamine on single cortical neurons. Brain Res. **423:** 347–352, 1987.

Erlander, M. G., Lovenberg, T. W., Baron, B. M., DeLecea, L., Danielson, P. E., Racke, M., Slone, A. L., Siegel, B. W., Foye, P. E., Cannon, K., Burns, J. and Sutcliffe, J. G.: Two members of a distinct subfamily of 5-hydroxytryptamine receptors differentially expressed in rat brain. Proc. Natl. Acad. Sci. U.S.A. **90:** 3452–3456, 1993.

Ferry, R. C., Unsworth, C. D. and Molinoff, P. B.: Effects of agonists, partial agonists, and antagonists on the regulation of 5-hydroxytryptamine$_2$ receptors in P11 cells. Mol. Pharmacol. **43:** 726–733, 1993.

Garratt, J. C., Alreja, M. and Aghajanian, G. K.: LSD has high efficacy relative to serotonin in enhancing the cationic current I_h: Intracellular studies in rat facial motoneurons. Synapse **13:** 123–134, 1993.

Geaney, D. P., Schachter, M., Elliot, J. M. and Grahame-Smith, D. G.: Characterization of [^{3}H]lysergic acid diethylamide binding to a 5-hydroxytryptamine receptor on human platelet membranes. Eur. J. Pharmacol. **97:** 87–93, 1984.

Gellman, R. L. and Aghajanian, G. K.: Pyramidal cells in piriform cortex receive a convergence of inputs from monoamine activated GABAergic interneurons. Brain Res. **600:** 63–73, 1993.

Gellman, R. L. and Aghajanian, G. K.: Serotonin$_2$ receptor-mediated excitation of interneurons in piriform cortex: Antagonism by atypical antipsychotic drugs. Neuroscience **58:** 515–525, 1994.

Gerald, C., Adham, N., Kao, H.-T., Olsen, M. A., Laz, T. M., Schechter, L. E., Bard, J. A., Vaysse, P. J.-J., Hartig, P. R., Branchek, T. A. and Weinshank, R. L.: The 5-HT$_4$ receptor: Molecular cloning and pharmacological characterization of two splice variants. EMJO J. **14:** 2806–2815, 1995.

Glennon, R. A.: Do classical hallucinogens act as 5-HT$_2$ agonists or antagonists? Neuropsychopharmacology **3:** 509–517, 1990.

Glennon, R. A., Titeler, M. and McKenney, J. D.: Evidence for 5-HT$_2$ involvement in the mechanism of action of hallucinogenic agents. Life Sci. **35:** 2505–2511, 1984.

Hawks, R. L. and Chiang, C. N.: Urine testing for drugs of abuse. National Institute on Drug Abuse, Research Monograph #73. U.S. Department of Health and Human Services, DHHS Publication No. (ADDM)87-1481, Rockville, MD, 1986.

Hoyer, D.: Functional correlates of serotonin 5-HT$_1$ recognition sites. J. Recept. Res. **8:** 59–81, 1988.

Kadan, M. J., Krohn, A. M., Evans, M. J., Waltz, R. L. and Hartig, P. R.: Characterization of ^{125}I-lysergic acid diethylamide binding to serotonin receptors in rat frontal cortex. J. Neurochem. **43:** 601–606, 1984.

Laubscher, A., Pletscher, A. and Noll, H.: Interaction of D-LSD with blood platelets of rabbits: Shape change and specific binding. J. Pharmacol. Exp. Ther. **216:** 385–389, 1981.

Lovenberg, T. W., Baron, B. M., de Lecea, L., Miller, J. D., Prosser, R. A., Rea, M. A., Foye, P. E., Racke, M., Slone, A. L., Siegel, B. W., Danielson, P. E., Sutcliffe, J. G. and Erlander, M. G.: A novel adenylyl cyclase-activating serotonin receptor (5-HT$_7$) implicated in the regulation of mammalian circadian rhythms. Neuron **11:** 449–458, 1993b.

Lovenberg, T. W., Erlander, M. G., Baron, B. M., Racke, M., Slone, A. L., Siegel, B. W., Craft, C. M., Burns, J. E., Danielson, P. E. and Sutcliffe, J. G.: Molecular cloning and functional expression of 5-HT$_{1E}$-like rat and human 5-hydroxytryptamine receptor genes. Proc. Natl. Acad. Sci. U.S.A. **90:** 2184–2188, 1993a.

Lyon, R. A., Davis, K. H. and Titeler, M.: ^{3}H-DOB (4-bromo-2,5-dimethoxyphenylisopropylamine) labels a guanyl nucleotide-sensitive state of cortical 5-HT$_2$ receptors. Mol. Pharmacol. **31:** 194–199, 1987.

Marek, G. J. and Aghajanian, G. K.: Excitation of interneurons in piriform cortex by 5-hydroxytryptamine (5-HT): Blockade by MDL 100,907, a highly selective 5-HT$_{2A}$ antagonist. Eur. J. Pharmacol. **259:** 137–141, 1994.

Marek, G. J. and Aghajanian, G. K.: α_{1B}-Adrenoreceptor mediated excitation of piriform cortical interneurons. Eur. J. Pharmacol., **305:** 95–100, 1996.

Marek, G. J. and Aghajanian, G. K.: Protein kinase C inhibitors enhance the 5-HT$_{2A}$ receptor-mediated excitatory effects of serotonin on interneurons in rat piriform cortex. Synapse **21:** 123–130, 1995.

Matthes, H., Boschert, U., Amlaiky, N., Grailhe, R., Plassat, J.-L., Muscatelli, F., Mattei, M.-G. and Hen, R.: Mouse 5-hydroxytryptamine$_{5A}$ and 5-hydroxytryptamine$_{5B}$ receptors define a new family of serotonin receptors: Cloning, functional expression, and chromosomal localization. Mol. Pharmacol. **43:** 313–319, 1993.

Mengod, G., Pompeiano, M., Martinez-Mir, M. I. and Palacios, J. M.: Localization of the mRNA for the 5-HT$_2$ receptor by in situ hybridization histochemistry. Correlation with the distribution of receptor sites. Brain Res. **524:** 139–143, 1990.

Monsma, F. J., Shen, Y., Ward, R. P., Hamblin, M. W. and Sibley, D. R.: Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. Mol. Pharmacol. **43:** 320–327, 1993.

Palfreyman, M. G., Schmidt, C. J., Sorensen, S. M., Dudley, M. W., Kehne, J. H., Moser, P., Gittos, M. W. and Carr, A. A.: Electrophysiological, biochemical and behavioral evidence for 5-HT$_2$ and 5-HT$_3$ mediated control of dopaminergic function. Psychopharmacology. **112:** suppl., S60–S67, 1993.

Pazos, A., Cortes, R. and Palacios, J. M.: Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Res. **346:** 231–249, 1985.

Peroutka, S. J. and Hamik, A.: [^{3}H]Quipazine labels 5-HT$_3$ recognition sites in rat cortical membranes. Eur. J. Pharmacol. **148:** 297–299, 1988.

Peroutka, S. J. and Snyder, S. H.: Multiple serotonin receptors: Differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]lysergic acid diethylamide and [^{3}H]spiroperidol. Mol. Pharmacol. **16:** 687–699, 1979.

Pierce, P. A. and Peroutka, S. J.: Antagonism of 5-hydroxytryptamine$_2$ receptor-mediated phosphatidylinositol turnover by d-lysergic acid diethylamide. J. Pharmacol. Exp. Ther. **247:** 918–925, 1988.

PIERCE, P. A. AND PEROUTKA, S. J.: d-Lysergic acid diethylamide differentially affects the dual actions of 5-hydroxytryptamine on cortical neurons. Neuropharmacology **29:** 705–712, 1990.

PIERCE, P. A. AND PEROUTKA, S. J.: Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex. Psychopharmacology **97:** 118–122, 1989.

POMPEIANO, M., PALACIOS, J. M. AND MENGOD, G.: Distribution of the serotonin 5-HT_2 receptor family mRNAs: Comparison between 5-HT_{2A} and 5-HT_{2C} receptors. Mol. Brain Res. **23:** 163–178, 1994.

RASMUSSEN, K. AND AGHAJANIAN, G. K.: Effects of hallucinogens on spontaneous and sensory-evoked locus coeruleus unit activity in the rat: Reversal by selective 5-HT_2 antagonists. Brain Res. **385:** 395–400, 1986.

RASMUSSEN, K. AND AGHAJANIAN, G. K.: Potency of antipsychotics in reversing the effects of a hallucinogenic drug on locus coeruleus neurons correlates with 5-HT_2 binding affinity. Neuropsychopharmacology **1:** 101–107, 1988.

RUAT, M., TRAIFFORT, E., LEURS, R., TARDIVEL-LACOMBE, J., DIAZ, J., ARRANGE, J.-M. AND SCHWARTZ, J.-C.: Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT_7) activating cAMP formation. Proc. Natl. Acad. Sci. U.S.A. **90:** 8547–8551, 1993.

SANDERS-BUSH, E. AND BREEDING, M.: Choroid plexus epithelial cells in primary culture: A model of 5-HT_{1C} receptor activation by hallucinogenic drugs. Psychopharmacology **105:** 340–346, 1991.

SANDERS-BUSH, E., BURRIS, K. D. AND KNOTH, K.: Lysergic acid diethylamide and 2,5-dimethoxy-4-methylamphetamine are partial agonists at serotonin receptors linked to phosphoinositide hydrolysis. J. Pharmacol. Exp. Ther. **246:** 924–928, 1988.

SCHREIBER, R., BROCCO, M. AND MILLAN, M. J.: Blockade of the discriminative stimulus effects of DOI by MDL 100,907 and the "atypical" antipsychotics, clozapine and risperidone. Eur. J. Pharmacol. **264:** 99–102, 1994.

SHELDON, P. W. AND AGHAJANIAN, G. K.: Serotonin (5-HT) induces IPSPs in pyramidal layer cells of rat piriform cortex: Evidence for the involvement of a 5-HT_2-activated interneuron. Brain Res. **506:** 62–69, 1990.

SHELDON, P. W. AND AGHAJANIAN, G. K.: Serotonin (5-HT) and norepinephrine (NE) induce IPSPs in pyramidal cells of the piriform cortex: Evidence for a 5-HT_2 and $\alpha_1$activated interneuron. Soc. Neurosci. Abstr. **14:** 215, 1988.

SHELDON, P. W. AND AGHAJANIAN, G. K.: Excitatory responses to serotonin (5-HT) in neurons of the rat piriform cortex: Evidence for mediation by 5-HT_{1C} receptors in pyramidal cells and 5-HT_2 receptors in interneurons. Synapse **9:** 208–218, 1991.

SORENSEN, S. M., KEHNE, J. H., FADAYEL, G. M., HUMPHREYS, T. H., KETTELER, H. J., SULLIVAN, C. K., TAYLOR, V. L. AND SCHMIDT, C. J.: Characterization of the 5-HT_2 receptor antagonist MDL 100907 as a putative atypical antipsychotic: Behavioral, electrophysiological and neurochemical studies. J. Pharmacol. Exp. Ther. **266:** 684–691, 1993.

TITELER, M., LYON, R. A. AND GLENNON, R. A.: Radioligand binding evidence implicates the brain 5-HT_2 receptor as a site of action for LSD and phenylisopropylamine hallucinogens. Psychopharmacology **94:** 213–216, 1988.

WRIGHT, D. E., SEROOGY, K. B., LUNDGREN, K. H., DAVIS, B. M. AND JENNES, L.: Comparative localization of serotonin$_{1A}$, $_{1C}$, and $_2$ receptor subtype mRNAs in rat brain. J. Comp. Neurol. **351:** 357–373, 1995.

ZGOMBICK, J. M., SCHECHTER, L. E., MACCHI, M., HARTIG, P. R., BRANCHEK, T. A. AND WEINSHANK, R. L.: Human gene S31 encodes the pharmacologically defined serotonin 5-hydroxytryptamine$_{1E}$ receptor. Mol. Pharmacol. **42:** 180–185, 1992.

Send reprint requests to: Gerard J. Marek, Yale School of Medicine, Department of Psychiatry, Connecticut Mental Health Center and the Ribicoff Research Facilities, 34 Park St., New Haven, CT 06508.