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The role of the 5-HT_{2A} and 5-HT_{2C} receptors in the stimulus effects of hallucinogenic drugs III: the mechanistic basis for supersensitivity to the LSD stimulus following serotonin depletion

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Abstract The present study was designed to determine the effects of *p*-chlorophenylalanine (PCPA) and *p*-chloroamphetamine (PCA) administration on (1) the levels of serotonin (5-hydroxytryptamine, 5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in rat brain, (2) the sensitivity of LSD-trained rats to the stimulus effects of LSD, and (3) the maximal levels of 5-HT_{2A} and 5-HT_{2C} receptor mediated phosphoinositide (PI) hydrolysis in rat brain. PCA and PCPA both produced a significant depletion of whole brain 5-HT and 5-HIAA concentrations. The depletion of serotonin with PCPA, but not PCA, resulted in supersensitivity of LSD-trained subjects to the stimulus effects of LSD. Neither PCPA nor PCA treatment altered the maximal level of 5-HT_{2A} receptor-mediated PI hydrolysis. However, PCPA, but not PCA, treatment resulted in a significant upregulation (46%, $P < 0.05$) of the maximal level of 5-HT_{2C} receptor mediated PI hydrolysis. These data suggest that upregulation of the 5-HT_{2C} receptor mediates the supersensitivity to LSD discriminative stimulus which follows the depletion of central nervous system serotonin by PCPA.

Key words Serotonin · Hallucinogens · LSD · *p*-Chloroamphetamine (PCA) · *p*-Chlorophenylalanine (PCPA) · Drug-induced stimulus control · Phosphoinositide hydrolysis · 5-HT_{2A} · 5-HT_{2c}

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Introduction

Drug-induced stimulus control has been applied to the study of hallucinogens for nearly 3 decades (for reviews see Appel et al. 1982; Glennon 1990). The integration of in vitro biochemical data with in vivo drug discrimination data has formed the foundation for our present understanding of the mechanisms by which hallucinogens elicit their unique interoceptive states in animal subjects. Several consistent observations have indicated that serotonin (5-hydroxytryptamine, 5-HT) receptors, in particular 5-HT_2 receptors, mediate the stimulus properties of the indolamine and phenylalkylamine hallucinogens (for reviews see Glennon 1994; Winter 1994).

Recently, the application of antagonist correlation analysis to the study of hallucinogen-induced stimulus control has demonstrated that agonist interactions with 5-HT_{2A} receptors, but not 5-HT_{2C} receptors, are prerequisite for the maintenance of LSD-induced stimulus control and for the substitution of (–)DOM for the LSD stimulus (Fiorella et al. 1995b,c). However, in vivo agonist interactions with 5-HT_{2A} receptors, and the ability to substitute for the LSD and (–)DOM stimuli are not characteristics unique to hallucinogens. The non-hallucinogenic congener of LSD, lisuride, as well as the non-hallucinogenic phenylpiperazine quipazine, both substitute for the discriminative stimuli of LSD, racemic DOM and (–)DOM (Glennon and Hauck 1985; Fiorella et al. 1995d). Taken together, these data indicate that 5-HT_{2A} agonist interactions may be required, but are not sufficient criteria for a given compound to elicit hallucinations in humans. For this reason, the determination of additional interactions which mediate the stimulus properties of hallucinogens is required to supplement the present understanding of the mechanisms of action of these compounds.

Agonist activity at the 5-HT_{2C} receptor has been hypothesized to play a role in the stimulus effects of hallucinogens. Indolamine and phenylalkylamine

hallucinogens possess both high affinity for and partial agonist activity at 5-HT_{2C} receptors (Sanders-Bush et al. 1988; Titeler et al. 1988). In addition, Burris et al. (1991) demonstrated that while LSD stimulates increases in PI turnover at 5-HT_{2C} sites, lisuride and BOL, non-hallucinogenic congeners of LSD, do not. Despite these intriguing findings, the 5-HT_{2C} receptor has not yet been firmly established as a mediator of the stimulus properties of hallucinogens.

The selective study of the role of the 5-HT_{2C} receptor in the stimulus effects of hallucinogens is confounded by several factors. (1) The predominant role of 5-HT_{2A} agonist activity in the maintenance of hallucinogen-induced stimulus control may obscure the roles of other less salient components of the hallucinogenic stimulus complex; (2) the marked lack of selectivity characteristic of available 5-HT_2 agonists and antagonists often precludes the differentiation of 5-HT_{2A} from 5-HT_{2C}-mediated effects (Sanders-Bush and Breeding 1988; Leysen 1990; Van Wijngaarden et al. 1990; Glennon and Dukat 1991; Sahin-Erdemli et al. 1991). For these reasons, novel approaches are required to selectively study the role of the 5-HT_{2C} receptor in hallucinogenic stimuli. One such approach is the investigation of those interactions which modulate the sensitivity of subjects to the stimulus effects of hallucinogens (Glennon et al. 1991).

The depletion of CNS serotonin has been demonstrated, in some cases, to produce supersensitivity to the stimulus effects of hallucinogens. 5,7-Dihydroxytryptamine (5,7-DHT), *p*-chlorophenylalanine (PCPA), and *p*-chloroamphetamine (PCA) have been demonstrated to produce selective depletions of central serotonin (Koe and Weissman 1966; Jequier et al. 1967; Bjorkland et al. 1975; Neckers et al. 1976; Harvey et al. 1978; Kohler et al. 1978). While these agents all produce analogous reductions in whole brain serotonin levels, each compound acts by a distinct mechanism and produces a distinct anatomical distribution of serotonin depletion. These differences might account for the differential effects of 5,7-DHT, PCPA, and PCA-induced serotonin depletion on the sensitivity of subjects to the LSD discriminative stimulus.

The depletion of CNS serotonin by 5,7-DHT and PCPA, but not PCA, has been reported to increase the sensitivity of animal subjects to the disruptive and stimulus effects of LSD (Joseph and Appel 1976, 1977; Commissaris et al. 1979; White et al. 1980). These behavioral data indicate that some effect secondary to 5,7-DHT and PCPA, but not PCA-induced serotonin depletion is responsible for the subsequent behavioral supersensitivity to LSD. Thus, the determination of a physiologic effect which follows both 5,7-DHT and PCPA, but not PCA treatments would offer important insight into the mechanistic basis for depletion-induced supersensitivity to LSD and correspondingly the mechanism of action of LSD.

Biochemical studies have demonstrated that 5,7-DHT-induced depletion of serotonin does not induce an upregulation in either the density of central 5-HT_{2A} receptors or 5-HT_{2A} receptor mediated PI turnover (Conn and Sander-Bush 1986). In contrast, lesions produced by 5,7-DHT have been reported to produce a significant upregulation in both the density of 5-HT_{2C} receptors (Pranzatelli 1990) and the PI turnover response mediated by the 5-HT_{2C} receptor (Conn et al. 1987). Taken together, these studies imply that an increase in 5-HT_{2C}, but not 5-HT_{2A} receptors, mediates the behavioral supersensitivity to the LSD stimulus which follows 5,7-DHT-induced serotonin depletion.

The present study was designed to test the hypothesis that upregulation of the 5-HT_{2C} receptor mediates supersensitivity to the LSD stimulus following 5-HT depletion. Specifically, experiments were designed to determine whether the behavioral and biochemical effects produced by PCA and PCPA treatments were consistant with this 5-HT_{2C} hypothesis of serotonin depletion-induced supersensitivity to LSD. The effects of PCPA and PCA administration on (1) the concentrations of 5-HT and 5-HIAA in rat brain, (2) the sensitivity of LSD-trained rats to the LSD discriminative stimulus in vivo, and (3) the maximal level of 5-HT_{2A} and 5-HT_{2C} receptor mediated PI turnover in vitro, were investigated.

Materials and methods

Behavioral experiments

Animals

Thirty-six Male Fischer 344 rats were obtained from Harlan Sprague-Dawley (Indianapolis, Ind.). They were housed in pairs under a natural light-dark cycle and allowed free access to water in the home cage. Subjects were fed immediately following experimental sessions. Caloric intake was controlled to yield an approximate mean body weight of 250 g.

Apparatus

Six small animal test chambers (Coulbourn Instruments Model E10-10) housed in larger light-proof, sound insulated boxes were used for all experiments. Each box had a house light and exhaust fan. The chamber contained two levers mounted on opposite ends of one wall. Centered between the levers was a dipper that delivered 0.1 ml sweetened condensed milk diluted 2:1 with tap water.

LSD-induced stimulus control procedure

Subjects were trained to discriminate LSD (0.1 mg/kg, 15-min pretreatment, IP injection) from saline as described previously (Fiorella et al. 1995b). A fixed ratio 10 (FR10) schedule of reinforcement was used. Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, 83% or more of all responses prior to the delivery of the first reinforcer were on the appropriate lever. Test sessions were initiated after stimulus control was firmly established.

Test procedure

During test sessions, no responses were reinforced and the session was terminated after the emission of ten responses on either lever. The distribution of responses between the two levers was expressed as the percentage of total responses emitted on the drug-appropriate lever. Response rate was calculated for each session by dividing the total number of responses emitted prior to lever selection, that is, prior to the emission of ten responses on either lever, by the elapsed time.

History of animals used in the present study

Subjects used in the present study had undergone 12 months of training sessions with intermittent test sessions. Data generated with these subjects have been presented previously (Fiorella et al. 1995b, c, d).

Treatment groups

Animal subjects were divided into two groups of 18; one was designated for the PCPA depletion study, the other for the PCA depletion study. In each group of 18, nine subjects were designated for either PCPA or PCA treatment, while the other nine were designated to serve as a parallel control group.

Pre-depletion study (phase 1)

The purpose of these experiments was to (1) assess the effect of a period of discontinued training followed by three consecutive test sessions on the LSD dose-response relationship and, (2) generate control data describing the dose-response relationship in subjects to be treated in phase 2 of the study. Table 1 outlines the schedule for phase 1 of the present study. First, during the training period, all subjects participated in LSD and saline training sessions on 14 consecutive days. Second, training sessions were discontinued for either 5 or 12 consecutive days for the PCPA and PCA experimental groups, respectively. The duration of the discontinuation period was identical to that required in phase 2 of the depletion study. Next, on 3 consecutive days, subjects were tested with either 0.015, 0.03, or 0.06 mg/kg LSD according to the test session methodology previously described. On each test day, three subjects from both the treatment and parallel control groups received each test dose. Therefore, after the three consecutive test sessions, each subject had been tested at each dose one time. Following these test sessions, all subjects were trained with 0.1 mg/kg LSD.

Serotonin depletion study (phase 2)

The purpose of this phase of the study was to assess the effects of either PCPA or PCA treatment on the sensitivity of LSD-trained subjects to the stimulus properties of LSD. Table 1 outlines the schedule for phase 2 of the present study. During the training period, 14 consecutive training sessions were conducted with LSD and saline. Next, during the treatment period, subjects were treated with the appropriate serotonin depleting agent or saline. Subjects in the PCPA group received 100 mg/kg PCPA by IP injection for 3 consecutive days, while the parallel control group received an equal volume of saline for 3 consecutive days. Subjects in the PCA group received 12.5 mg/kg chlorpromazine followed 30 min later by 10 mg/kg PCA, both by IP injection. The parallel PCA control group received 12.5 mg/kg chlorpromazine, followed by an injection of saline. No training sessions were conducted during either the treatment period or the following post-treatment period. The regimen of treatment, as well as the duration of the post-treatment period were selected on the basis of previous behavioral and biochemical studies which have employed PCPA and PCA as serotonin depleting agents (Cameron and Appel 1973; Browne and Ho 1975; Appel et al. 1977; White et al. 1980; Schechter 1991). Following the post-treatment period, test sessions were conducted as described above for the phase 1 experiments.

Table 1 Schedule for phase 1 and phase 2 of the behavioral experiment

	Days PCPA/Control$_1$	PCA/Control$_2$
A. Phase 1: pre-depletion		
Training period	1–14	1–14
Discontinuation of training	15–19	15–26
Test Period	20–22	27–29
Training session (0.1 mg/kg LSD)	23	30
B. Phase 2: serotonin depletion		
Training period	1–14	1–14
Treatment period	15–17	15–16
Post-treatment period	18–19	17–26
Test period	20–22	27–29
Training session (0.1 mg/kg LSD)	23	30

Biochemical experiments

Subjects for phosphoinositide hydrolysis (PI) experiments

Four parallel groups of subjects were used in PI hydrolysis experiments. The PCPA group received injections of 100 mg/kg PCPA for three consecutive days, while the PCPA control group received injections of saline. On day 3 following the final PCPA or saline injection, these rats were killed and used for PI measurements. The PCA group received injections of 12.5 mg/kg chlorpromazine followed 30 min later by 10 mg/kg PCA for 2 consecutive days; the PCA control group received 12.5 mg/kg chlorpromazine followed by an injection of saline. On day 10 following the last injection, these rats were killed and used for PI hydrolysis measurements.

Measurement of phosphoinositide (PI) hydrolysis

Serotonin stimulated phosphoinositide hydrolysis was measured using a modification of the method of Conn and Sanders-Bush (1986). Subjects were killed by decapitation, and frontal cortex and choroid plexus were dissected. Immediately following dissection, the tissues were submerged in calcium-free Krebs bicarbonate buffer (KRBG) (126 mM NaCl, 3.0 mM KCl, 1.4 mM KH_2PO_4, 1.3 mM $MgSO_4$, 25 mM $NaHCO_3$, 5.8 mM glucose; pH 7.4) which had previously been warmed to 37°C and thoroughly oxygenated with a gas mixture of 95% O_2-5% CO_2. Frontal cortex was then chopped into 0.35 mm × 0.35 mm slices with a McIlwain Tissue Chopper (Brinkman Instruments, Westbury, N.Y.). Frontal cortical slices and choroid plexus were then incubated at 37°C with calcium-free KRBG for 30 min under an atmosphere of 95% O_2-5% CO_2 at 37°C. Buffer was changed and reoxygenated every 5 min during this preincubation period. Following the preincubation, tissues were washed once with warm, oxygenated, calcium-containing KRBG (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 5.8 mM glucose; pH 7.4). All subsequent steps employed warm, oxygenated calcium-containing

KRBG. Tissues were labelled during a 60-min (frontal cortex) or 45-min (choroid plexus) incubation with 10 μCi (^{3}H)myo-inositol per ml of KRBG. After labelling, tissues were washed four times with 20 ml KRBG containing 5 mM myo-inositol, and then incubated for 2 min with 10 mM LiCl and 5 mM myo-inositol in KRBG. Gravity packed frontal cortical slices (50 μl) were then transferred to tubes containing 10 mM LiCl, 20 μM clorgyline, and either 250 μM 5-HT or buffer and incubated for 60 min at 37°C. Single choroid plexus was added to a tube containing 10 mM LiCl, 20 μM pargyline, and either 10 μM 5-HT or buffer and incubated for 30 min at 37°C. The final incubation volume was 300 μl. The incubation was terminated with 900 μl of an ice-cold 1:2 chloroform/methanol solution, followed by an additional 300 μl chloroform and 300 μl water. Tubes were mixed thoroughly and stored overnight at 4°C. The 5-HT dose-response experiments employed a slightly modified protocol: labelling times were 120 min and 60 min for cortex and choroid plexus, respectively, and stimulations were conducted in the presence of various concentrations of 5-HT. Labelling times were decreased in the depletion study to preserve the integrity of tissues better following chemical treatment (Sanders-Bush et al. 1986).

On the following day, samples were centrifuged at 1500 *g* for 15 min, and 600 μl of the upper aqueous phase was applied to Dowex-1 anionic-exchange resin (formate form). Inositol phosphates were eluted by the method of Berridge et al. (1982). Briefly, the columns were washed with 16 ml of 5 mM myo-inositol, followed by 8 ml of a 5 mM sodium tetraborate/60 mM ammonium formate solution. A final wash with 10 ml of a 1.0 M ammonium formate/0.1 M formic acid solution was collected. A 2-ml aliquot of this eluant was combined with 15 ml Liquiscint scintillation cocktail (National Diagnostics) and 2 drops of glacial acetic acid. Vials were incubated overnight, and radioactivity was measured by liquid scintillation spectrophotometry. The remaining organic and aqueous layers were dried under a steady stream of air. The tissue residue was then resuspended in 0.1 N NaOH and heated to 90°C for 30 min. The protein content of each sample was then determined by the method of Lowry et al. (1951). Average protein values were 2.53 (±0.098) mg per 50 μl cortex slices, and 0.094 (±0.007)mg per choroid plexus, respectively.

HPLC analysis of 5-HT and 5-HIAA levels

Three parallel groups of five subjects were treated with either saline (3 consecutive days), PCA (2 consecutive days, 12.5 mg/kg chlorpromazine followed 30 min later by 10 mg/kg PCA) or PCPA (3 consecutive days, 100 mg/kg). Two days after the last saline and PCPA injection and 10 days after the last PCA injection, rats were killed by decapitation, and brains were removed. Cerebellum and brain stem were removed and brains were hemisected along the cerebral longitudinal fissure. Half-brains were homogenized in 33.6 mM $HClO_4$ in water. The homogenates were centrifuged at 40000 *g* for 30 min. The supernates were diluted 1:10 in water and stored overnight at –20°C, and the corresponding protein pellets were digested in 0.1 N NaOH.

The concentrations of 5-HT and 5-HIAA in supernates were measured using HPLC-EC by the method of Lorrain and Hull (1993). Briefly, compounds in a 0.5 μl injection volume were separated in an LC packings-Fuscia II column, with a mobile phase consisting of 32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM sodium EDTA, 0.215 octyl sodium sulfate, and 3% methanol (pH 4.1). A Gilson model 307 pump, equipped with an Acurate flow splitter (70:1) and three pulse dampers, operated at a flow rate of 0.55 ml/min. 5-HT and 5-HIAA were detected with an Antec micro-EC controller (sensitivity at 0.02 nA/V), using a glassy carbon electrode maintained at a potential of +0.70 V relative to a Ag/AgCl reference electrode. Peak areas were integrated and concentrations were determined using external 5-HT and 5-HIAA standards. The protein content of each sample was determined by the method of Lowry et al. (1951).

Data analysis

Behavioral experiments

Two types of comparisons were made for each of the 5-HT depletion experiments. First, the performance of the treated subjects was compared to that of the parallel controls at each dose by individual applications of Wilcoxon's signed ranks test. Second, the performance of the treated subjects (phase 2 experiments) was compared to the performance of those same subjects during the test sessions which preceded treatments (phase 1 experiments). Comparisons were made at each dose by individual applications of the paired Wilcoxon's signed ranks test. Significant differences were indicated by P values of less than 0.05.

Biochemical experiments

Three replicate measurements of basal and stimulated (incubation with 250 μM 5-HT) phosphoinositide hydrolysis were performed with cortical slices from each experimental subject. One choroid plexus from each experimental subject was used for the measurement of basal and stimulated (incubation with 10 μM 5-HT) phosphoinositide hydrolysis, respectively. The cpm for each replicate was normalized to its protein content. Data were expressed as percent stimulation of phosphoinositide hydrolysis above basal [100 × (stimulated cpm/mg protein – basal cpm/mg protein)/basal cpm/mg protein]. Comparisons between the parallel control and treated groups were performed by application of Student's t-test. Significance was indicated by P values less than 0.05.

HPLC experiments

The mean values for 5-HT and 5-HIAA concentrations (ng/mg) in control, PCA, and PCPA treated subjects were compared using the Student-Newman-Keuls test.

Results

5-HT and 5-HIAA levels

The concentrations of 5-HT in the whole brains of subjects treated with PCA and PCPA were reduced to 62.2% ($P < 0.01$) and 54.2 ($P < 0.01$)% of the control values, respectively. Concentrations of 5-HIAA were reduced in PCA and PCPA treated subjects to 24.3% ($P < 0.01$) and 5.9% ($P < 0.01$) of control values, respectively. No difference was observed in the magnitude of the 5-HT or 5-HIAA depletions produced by the PCPA and PCA treatments (Table 2).

Table 2 Effect of PCPA and PCA on whole brain concentrations of 5-HT and 5-HIAA. Concentrations (ng/mg) represent the means of five individual determinations. Standard errors are expressed in parentheses

	Control	PCA	PCPA
5-HT	9.32(0.53)	5.80(0.40)*	5.05(0.193)*
5-HIAA	3.14(0.27)	0.763(0.13)*	0.186(0.031)*

*Indicates significant difference from the control values by the Student-Newman-Keuls test ($P = 0.01$)

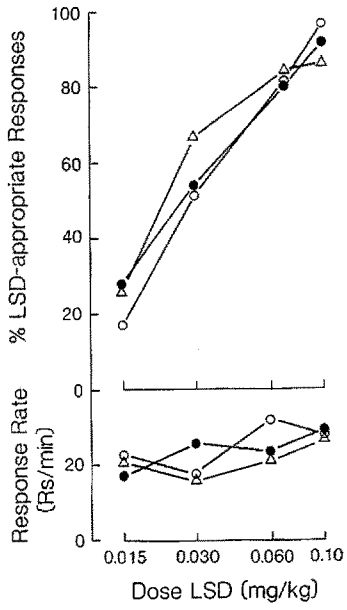

Fig. 1 LSD dose-response relationships prior to serotonin depletion. *Open circles* represent results from eight subjects tested within the normal context of training sessions. *Closed circles* represent results from the 18 subjects of the PCPA and parallel control groups. *Open triangles* represent results from the PCA and parallel control groups. PCPA, PCA and matched control groups were trained and tested as described in Table 1. *Abscissa*: dose of LSD (mg/kg); *Ordinate*: percentage of responses emitted on the LSD-appropriate lever

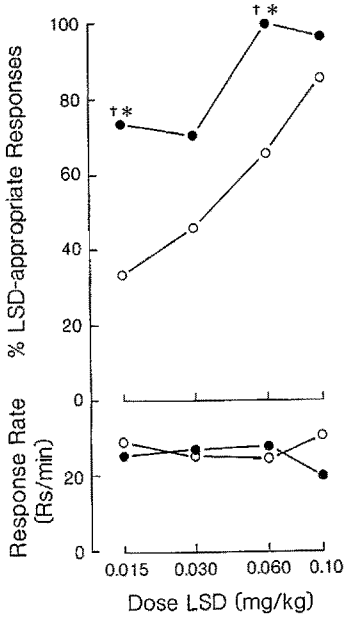

Fig. 2 The effect of PCPA-induced serotonin depletion on the LSD dose-response relationship. *Closed circles* represent results from nine subjects following treatment with PCPA. *Open circles* represent results from the nine parallel control subjects. Subjects were trained and tested as described in Table 1. *Abscissa*: dose LSD (mg/kg); *Ordinate*: percentage of responses emitted on the LSD-appropriate lever. *Indicates significant difference from parallel control ($P < 0.05$, Wilcoxon's signed ranks test), *cross* represents significant difference from the same subjects prior to PCPA treatment ($P < 0.05$, paired application of Wilcoxon's signed ranks test)

Behavioral experiments

Prior to serotonin depletion, the effects of the discontinuation of training on the stability of LSD-induced stimulus control were assessed (phase 1 experiments). No differences were detected between groups tested within the normal regimen of daily training sessions, after 5 consecutive days with no training sessions, or after 12 days with no training sessions (Fig. 1).

The LSD dose-response relationship was shifted to the left in the subjects treated with PCPA. PCPA treated subjects emitted significantly more LSD-appropriate responding after treatment with 0.015 and 0.06 mg/kg LSD than either (a) the parallel control group, (Fig 2) or (b) the same subjects prior to PCPA treatment.

In contrast, PCA treatment did not result in a shift of the LSD dose-response relationship (Fig. 3).

Biochemical experiments

Serotonin elicited dose-related increases in the accumulation of (^{3}H)inositol phosphates (IP) in both frontal cortical slices (5-HT_{2A} receptor mediated) and choroid plexus (5-HT_{2C} receptor mediated) (Fig. 4). On the basis of these dose-response studies, 250 μM and 10 μM concentrations of 5-HT were chosen to measure the maximal levels of [^{3}H]IP generation in cortical slices and choroid plexus, respectively.

5-HT_{2A}-mediated phosphoinositide hydrolysis was not altered following treatment with either PCPA or

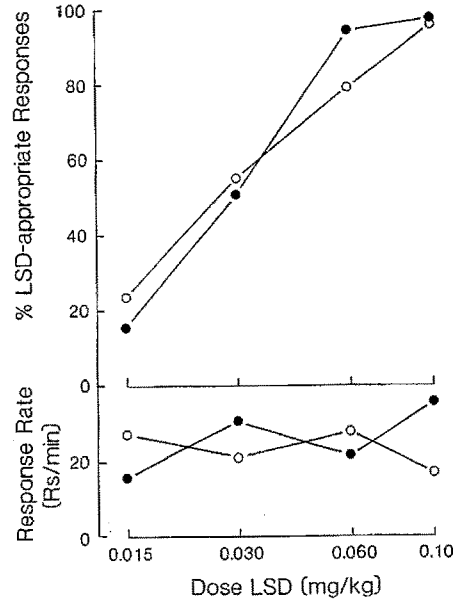

Fig. 3 The effect of PCA-induced serotonin depletion on the LSD dose-response relationship. *Closed circles* represent results from nine subjects following treatment with PCA. *Open circles* represent results from the nine parallel control subjects. Subjects were trained and tested as described in Table 1. *Abscissa*: dose of LSD (mg/kg); *Ordinate*: percentage of responses emitted on the LSD-appropriate lever. Other details are as in Fig. 2

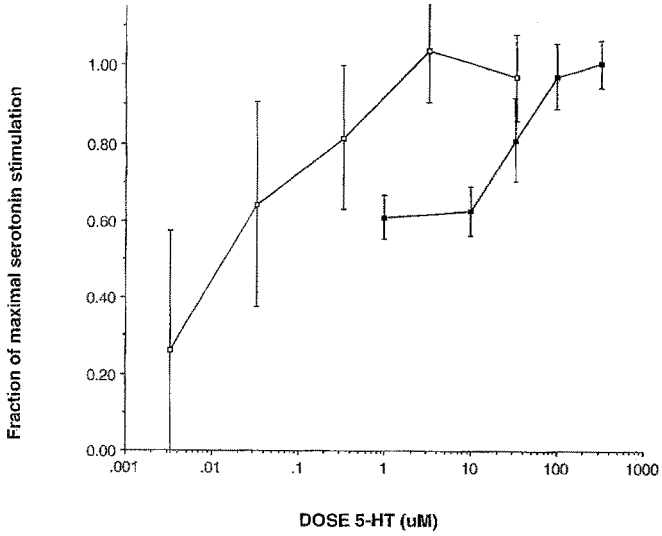

Fig. 4 Effect of 5-HT on the accumulation of (^{3}H)IP in frontal cortex slices (*closed squares*) and choroid plexus (*open squares*). (^{3}H)inositol labelled cortical slices and choroid plexus were incubated with 10 mM LiCl, 10 μM clorgyline (frontal cortex) or 10 μM pargyline (choroid plexus), and increasing concentrations of 5-HT. Each point represents the mean of five to seven individual determinations. The level of basal (^{3}H)IP generated was 1330 (±178) cpm/mg protein and 14 000 (±3600) cpm/mg protein in cortical slices and choroid plexus, respectively. Maximal percent stimulation of PI hydrolysis above basal was 61.2% (±3.5) and 752% (±70) in cortical slices and choroid plexus, respectively. *Ordinate*: fraction of maximal response; *abscissa*: dose of 5-HT (micromolar)

PCA. 5-HT_{2C} mediated phosphoinositide hydrolysis was 46% greater ($P < 0.05$) in the choroid plexus of the PCPA treated subjects than in those of the parallel control group. In contrast, PCA treatment did not result in any significant change in 5-HT_{2C} mediated PI hydrolysis (Table 3).

Discussion

The different regulatory behaviors exhibited by 5-HT_{2A} and 5-HT_{2C} receptors in response to serotonin depletion represent one means by which to distinguish the respective roles of these two receptor systems (Berendsen et al. 1991). Numerous studies have consistently demonstrated the 5-HT_{2A} receptor to be refractory to neurotransmitter depletion-induced upregulation (Blackshear et al. 1981; Quick and Azmitia 1983; Hall and Wedel 1985; Conn and Sanders-Bush 1986). In contrast, the 5-HT_{2C} receptor has been demonstrated to display the expected upregulation in response to 5,7-DHT-induced serotonin depletion (Conn et al. 1987; Pranzatelli; 1990).

The application of neurotransmitter depletion methods to the study of hallucinogens dates back to the earliest investigations of these agents. Original hypotheses describing the mechanism of action of LSD were based on biochemical studies which demonstrated that LSD diminished the release of 5-HT from pre-synaptic neurons and suppressed their basal level of electrical activity (Freedman 1961; Aghajanian et al. 1968). However, the expectation that the depletion of serotonin would counteract the in vivo effects of LSD was not fulfilled. Instead, a consistent supersensitivity to the behavioral effects of LSD was observed following the depletion of CNS serotonin (Appel and Freedman 1964; Appel et al. 1970a,b). In addition, these behavioral observations in animals have interesting correlates in humans (for review see Jacobs 1987). For example, humans are supersensitive to the psychotropic effects of LSD following reserpine treatment (Resnick et al. 1965). These findings suggest that (1) LSD exerts its effects primarily through post-synaptic, rather than pre-synaptic, interactions, and (2) the population of post-synaptic receptors which mediates the psychotropic effects of LSD is susceptible to upregulation following the depletion of serotonin.

In the present study, serotonin depletion produced by PCPA and PCA (Table 2) had differential effects on the sensitivity of LSD trained subjects. Although both agents produced analogous decreases in whole brain serotonin, PCPA treatment increased the sensitivity of LSD-trained subjects to the LSD stimulus (Fig. 2), while PCA had no effect (Fig. 3). Thus, it is not simply the depletion of CNS 5-HT which mediates supersensitivity to the LSD stimulus. Rather, some change which specifically occurs following PCPA-induced 5-HT depletion, but not PCA-induced 5-HT depletion, mediates supersensitivity to the LSD stimulus.

The mechanism and pattern of serotonin depletion produced by PCPA and PCA are different (White et al. 1980). PCPA irreversibly inhibits tryptophan hydroxylase, the enzyme responsible for the synthesis of 5-HT (Koe and Weissman 1966). This inhibition results in a gradual decline of serotonin levels throughout the CNS including the B7 and B8 cell groups of the raphe nuclei (Joseph and Appel 1977; Meek 1978). This pattern of

Table 3 5-HT_{2A} and 5-HT_{2C} mediated phosphoinositide hydrolysis in response to supramaximal (250 μM and 10 μM, respectively) concentrations of 5-HT. Responses are expressed in percent stimulation above basal (100 × (stimulated–basal)/basal). The reported values are the averages of seven to ten individual determinations. Average levels of basal (^{3}H)IP formation in the choroid plexus were 13 700 (± 1600) and 12 000 (± 1900) cpm/mg protein for the PCPA and PCA experiments, respectively. Average levels of basal (^{3}H)IP formation in the cortex were 700 (± 42) and 640 (± 50) cpm/mg protein for the PCPA and PCA experiments, respectively

Receptor (tissue)	Percent stimulation above basal Control$_1$	PCPA	Control$_2$	PCA
5-HT_{2A} (frontal cortex)	66.1(6.1)	67.3(7.6)	71.0(2.6)	67.5(8.4)
5-HT_{2C} (choroid plexus)	654(82)	960(111)*	739(144)	796(78)

*Indicates significant difference from parallel control value ($P < 0.05$) by Student's *t*-test

serotonin depletion is similar to that produced by 5,7-DHT (Fuxe et al. 1978). In contrast, PCA produces a pattern of neurodegeneration and cell death which has a more selective distribution within the B9 group of raphe nuclei (Bertillson 1975). The present study was designed to determine whether these mechanistic and anatomical differences are manifest in differential effects on the 5-HT_2 serotonergic systems which in turn might account for the corresponding effects on the behavioral sensitivity to the stimulus effects of LSD.

PCPA depletion, but not PCA depletion, elicited increases in the maximal level of 5-HT_{2C} receptor mediated phosphoinositide hydrolysis. No change in 5-HT_{2A} receptor mediated PI hydrolysis was observed after either treatment. These data indicate that upregulation of the 5-HT_{2C} receptor may mediate the supersensitivity to the LSD stimulus elicited by PCPA administration. Correspondingly, PCA-induced 5-HT depletion, which does not result in an upregulation of the 5-HT_{2C} receptor, does not result in an increased sensitivity to the LSD stimulus.

Thus, the biochemical and behavioral effects of PCPA closely resemble those of 5,7-DHT. 5,7-DHT depletes CNS serotonin (Baumgarten 1973), elicits an increased sensitivity to the LSD stimulus (White et al. 1980), and elicits an upregulation of the 5-HT_{2C} receptor independent of any change in 5-HT_{2A} receptor sensitivity (Conn and Sanders-Bush 1986; Conn et al. 1987; Pranzetelli; 1990). Taken together, these data further support the hypothesis that an upregulation of the 5-HT_{2C} receptor mediates supersensitivity to the LSD stimulus. When considered in the context of previous studies which have demonstrated that agonist interactions with the 5-HT_{2A} receptors predominantly mediate the stimulus properties of hallucinogens (Ismaiel et al. 1993; Schreiber et al. 1994; Fiorella et al. 1995c), the present data are consistent with the hypothesis that agonist interactions with 5-HT_{2C} receptors play a modulatory role with respect to the stimulus properties of hallucinogenic agents (Glennon et al. 1991).

The biochemical data presented here have further implications for other studies which have investigated the effects of PCPA treatment on the sensitivity of trained subjects to various discriminative stimuli. For example, PCPA administration has been demonstrated to elicit increases in sensitivity to the mescaline (Browne and Ho 1975), quipazine (White et al. 1979) and mCPP (Callahan and Cunningham 1994) stimuli, but not to the *d*-amphetamine (Schechter 1991), 8-OH-DPAT (Kalkman 1990), phencyclidine (Jarbe et al. 1975), nicotine (Schechter and Rosecrans 1972), fentanyl (Colpaert et al. 1977), MDMA, MDE or fenfluramine stimuli (Schechter 1991). Those stimuli which are more salient following PCPA administration may be mediated in part by interactions with 5-HT_{2C} receptors. This hypothesis is of particular interest with respect to the existing data describing the behavioral effects of mCPP. mCPP has been observed to be a partial agonist with respect to the stimulation of PI hydrolysis at the 5-HT_{2C} receptor (Conn and Sanders-Bush 1987; Fiorella et al., unpublished observations). Winter and Rabin (1993) and Callahan and Cunningham (1994) demonstrated that various agents reported to function as 5-HT_{2C} agonists mimic the stimulus effects of mCPP. In addition, the application of antagonist correlation analysis has indicated that the mCPP stimulus is mediated in part by agonist interactions with 5-HT_{2C} receptors (Fiorella et al. 1995a). The increases in sensitivity to the mCPP stimulus (Callahan and Cunningham 1994), as well as mCPP-induced hypolocomotion (Lucki et al. 1991) and mCPP-induced penile erections (Berendsen et al. 1991), which have been reported to follow PCPA-induced serotonin depletion can now be interpreted as further indications that 5-HT_{2C} agonist interactions mediate the behavioral effects of mCPP.

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