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Serotonergic/glutamatergic interactions: Potentiation of phencyclidine-induced stimulus control by citalopram

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Abstract

Previous investigations in our laboratory have found that the stimulus effects of the hallucinogenic serotonergic agonists DOM and LSD are potentiated by phencyclidine [PCP], a non-competitive NMDA antagonist. Also suggestive of behaviorally significant serotonergic/glutamatergic interactions is our finding that stimulus control by both PCP and LSD is partially antagonized by the mGlu2/3 agonist, LY 379268. These observations coupled with the fact that the stimulus effects of LSD and DOM are potentiated by selective serotonin reuptake inhibitors [SSRIs] led us in the present investigation to test the hypothesis that stimulus control by PCP is potentiated by the SSRI, citalopram. Stimulus control was established with PCP [3.0 mg/kg; 30 min pretreatment time] in a group of 12 rats. A two-lever, fixed ratio 10, positively reinforced task with saline controls was employed. Potentiation by citalopram of an intermediate dose of PCP was observed. In an attempt to establish the mechanism by which citalopram might interact with PCP, subsequent experiments examined the effects on that interaction of antagonists at serotonergic receptors. It was found that the selective 5-HT_{2C}-selective antagonists, SDZ SER 082 and SB 242084, significantly, albeit only partially, blocked the effects of citalopram on PCP. In agreement with our previous conclusions regarding the interaction of citalopram with DOM, the present data suggest that potentiation of the stimulus effects of PCP by citalopram are mediated in part by agonist activity at 5-HT_{2C} receptors.

Keywords: Drug discrimination; Phencyclidine; Citalopram; SB 242084; SDZ SER 082; Rat

1. Introduction

Previous studies in our laboratory have found that the stimulus effects in rats of both indoleamine and phenethylamine hallucinogens are augmented by the co-administration of the selective serotonin reuptake inhibitors fluoxetine, fluvoxamine, and venlafaxine [SSRIs] (Fiorella et al., 1996; Winter et al., 1999a, 2002). These findings are in general agreement with an anecdotal report of an increase in the effects of lysergic acid diethylamide [LSD] in an individual who co-administered fluoxetine in an attempt to augment the LSD experience (Bonson and Murphy, 1996) and of LSD flashbacks in persons with a history of LSD abuse subsequently treated with SSRIs (Markel et al., 1994). Interpretation of the animal data is confounded by the fact that SSRIs may partially mimic the stimulus effects of the phenethylamine hallucinogen, [−]-2,5-dimethoxy-4-methylamphetamine [DOM] (Winter et al., 1999b) or may inhibit the metabolism of DOM (Eckler et al., 2002). However, these interpretational problems are largely overcome by the use of citalopram, an SSRI which appears to be truly selective for the serotonin transporter (Bymaster et al., 2002; Hyttel, 1994; Milne and Goa, 1991) and which neither mimics the stimulus effects of DOM nor alters its metabolism (Eckler et al., 2002).

On the basis of reports that phencyclidine [PCP] and dizocilpine, non-competitive antagonists of the NMDA subtype of ionotropic glutamate receptors, increase serotonin levels in rat brain (Yan et al., 1997; Martin et al., 1998)

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we reasoned that NMDA antagonists might potentiate the stimulus effects of hallucinogens in a fashion similar to that of the SSRIs. Indeed, it subsequently was found that stimulus control by both DOM (Winter et al., 2000a) and LSD (Winter et al., 2004) is potentiated by PCP. Thus having observed potentiation of serotonergic hallucinogens by serotonergic agents, the SSRIs, and by an NMDA antagonist, PCP, the present investigation tested the symmetry of these serotonergic/glutamatergic interactions by examining the effects of citalopram in rats trained with PCP as a discriminative stimulus. Furthermore, on the basis of a previous investigation which concluded that the effects of citalopram on stimulus control by DOM are partially mediated by 5-HT_{2C} receptors (Eckler et al., 2004), we tested the effects of selective serotonergic antagonists.

2. Materials and methods

2.1. Subjects

A group of 12 male Fischer 344 rats was obtained at an age of approximately 6 weeks from Harlan Sprague–Dawley Inc. [Indianapolis, IN, U.S.A.], housed in pairs under a 12-h light–dark cycle beginning at 6:00 a.m., and allowed free access to water in their home cages. All training and testing took place during the light cycle. Caloric intake was controlled to maintain a mean body weight of approximately 275 g. Subjects were fed standard rat chow following experimental sessions. Caloric control has been shown to lengthen the life span and decrease the incidence of a variety of pathologies in Fischer 344 rats (Keenan et al., 1994). Based on a recent sample of 25 rats, the average life span under these conditions is 34.3 months [S.E.M. = 1.1]. Animals used in these studies were maintained in accordance with U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals as amended August 2002. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University at Buffalo.

2.2. Discrimination training

Six small animal test chambers [MED Associates ENV-008] were used for all experiments. These were housed in larger light-proof, sound-insulated boxes which contained a house light and an exhaust fan. Chambers contained two levers mounted at opposite ends of one wall. Centered between the levers was a dipper which delivered 0.1 ml of sweetened condensed milk diluted 2:1 with tap water. Sessions were managed by a micro-computer using operant control software [MED-PC State Notation, Version IV].

After learning to drink from the dipper, rats were trained to press first one and then the other of the two levers. The number of responses for each reinforcement was gradually increased from 1 to 10. During this time, the reinforced lever was alternated on a random basis. All subsequent training and testing sessions used a fixed-ratio 10 [FR10] schedule of reinforcement. Discrimination training was then begun. Subjects were trained to discriminate PCP [3.0 mg/kg, 30 min pretreatment time, IP; $N = 12$] from saline as described previously (Hirschhorn and Winter, 1971; Fiorella et al., 1995a). Following the administration of PCP, every tenth response on the PCP-appropriate lever was reinforced. Similarly, responses on the saline-appropriate lever were reinforced on a FR10 schedule following the injection of saline. For half of the subjects, the left lever was designated as the PCP-appropriate lever. During discrimination training, PCP and saline were alternated on a daily basis. PCP-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, i.e., no more than 2 incorrect responses prior to completion of the FR10 on the correct lever.

2.3. Test procedures

After stimulus control with PCP was well established, tests of generalization were conducted once per week in each animal. Tests were balanced between subjects trained on the previous day with saline and PCP, respectively. During test sessions, no responses were reinforced and the session was terminated after the emission of 10 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 total number of responses emitted prior to lever selection, that is, prior to the emission of 10 responses on either lever, by elapsed time. Data for any subjects failing to emit 10 responses within the constraints of the 10-min test session were not considered in the calculation of the percent drug-appropriate responding but were included in the analysis of response rates. For purposes of discussion of these data, an intermediate degree of generalization or antagonism is defined as being present when the mean response distribution after a test drug or combination of drugs is less than 80% drug-appropriate and is statistically significantly different from the results following both training conditions.

The effects of citalopram on PCP-induced stimulus control were assessed by co-administration of citalopram [3.0 mg/kg, 90 min pretreatment] and PCP [30 min before testing]. The interactions of serotonergic antagonists with the effects of citalopram on PCP-induced stimulus control were assessed in experiments in which the antagonists were administered in combination with citalopram and PCP.

2.4. Drugs

The following drugs were generously provided by the organizations indicated: PCP HCl [National Institute on Drug Abuse, Rockville, MD, USA], racemic citalopram

hydrobromide [H. Lundbeck A/S, Copenhagen, Denmark], SB 242084 [GlaxoSmithKline, Great Britain]. The following were purchased from the commercial sources indicated: *m*-chlorophenylpiperazine [*m*CPP] and pirenperone [Sigma-Aldrich USA], SDZ SER 082 and WAY-100635 [Tocris, USA]. A stock solution of pirenperone [1 mg/ml] was made in a minimal volume of a 45% w/v aqueous solution of 2-hydroxy-propyl-β-cyclodextrin and solutions for injection were made by diluting the stock with sterile 0.9% NaCl. M100907 was synthesized at the Laboratory of Medicinal Chemistry, National Institute of Diabetes, Digestive and Kidney Disorders at the National Institutes of Health [Bethesda, MD]. A stock solution of M100907 [0.5 mg/ml] was made by dissolving M100907 in a minimal volume of 0.2% w/v tartaric acid and diluting with water. All other drugs were dissolved in 0.9% saline. Doses are expressed as mg/kg of the salts. The IP route was employed for all drugs with the exception of WAY-100635 which was administered SC. An injection volume of 1 ml/kg body weight was employed for all drugs.

2.5. Statistical analysis

The statistical significance of the interaction between citalopram and the stimulus effects of PCP was determined using 2-way repeated measures ANOVA with dose of PCP and treatment with citalopram as factors. For assessment of the statistical significance of antagonism by various drugs of the potentiation of PCP by citalopram, 1-way repeated measures ANOVA compared the results of PCP alone, PCP+citalopram, and PCP+citalopram+antagonist. Pairwise comparisons following ANOVA were made using the Holm–Sidak method. Differences were considered to be statistically significant if the probability of their having arisen by chance was <0.05. All analyses were conducted using SigmaStat 3.0 for Windows™ [Jandel Scientific Software, San Rafael, CA]. Control data were repeated for each comparison and statistical analyses were applied using the appropriate control sessions. However, for purposes of clarity, mean values for control data are shown in all figures.

3. Results

3.1. Potentiation of PCP by citalopram

Preliminary experiments examined the time course of interaction of citalopram [3 mg/kg] with an intermediate dose of PCP [1.0 mg/kg]. Although the stimulus effects of PCP were enhanced using pretreatment times as brief as 15 min, maximum enhancement occurred using a pretreatment time of 90 min and all subsequent experiments used that pretreatment time.

Fig. 1 shows an orderly dose-related increase in PCP-appropriate responding in rats trained and tested with PCP. When the same doses were tested in rats pre-treated with a fixed dose of citalopram, PCP-appropriate responding increased for all doses of PCP less than the training dose. For PCP doses of 0.6 and 1.0 mg/kg, two-way repeated measures ANOVA revealed a significant increase in PCP-appropriate responding following the combination of citalopram and PCP compared with PCP alone [$F(1,9)=59.31$; $p=0.001$]. Response rates were not altered by pretreatment with citalopram.

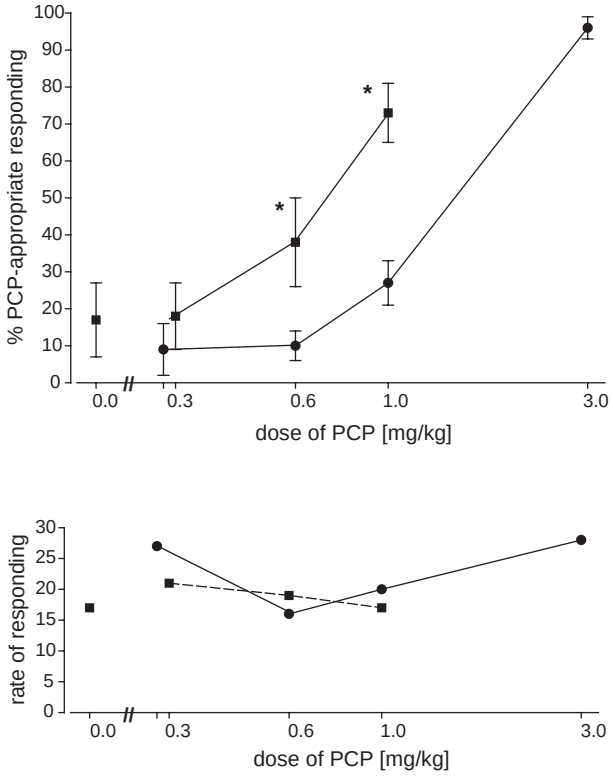

Fig. 1. Dose–response relationship for PCP alone and in combination with citalopram. (●) Effects of PCP alone in rats trained with PCP as a discriminative stimulus [3.0 mg/kg; 30 min pretreatment time]. (■) Effects of PCP in combination with citalopram [3.0 mg/kg; 90 min pretreatment time]. Each point represents the mean of one determination in each of 10 rats with the exception of the training dose where the mean of 4 determinations in each of the subjects is shown. Standard errors of the means are indicated. *Statistically significant difference between PCP alone and in combination with citalopram. The point at a dose of 0.0 is for citalopram alone. *Ordinate: Upper panel:* percent PCP-appropriate responding. *Lower panel:* rate expressed as responses per minute. *Abscissa:* dose plotted on a log scale.

3.2. Antagonism of the potentiation of PCP by citalopram

In Fig. 2 are shown the results of tests of interactions between a series of serotonergic antagonists in combination with an intermediate dose of PCP [1.0 mg/kg] following pretreatment with citalopram. It is seen that the selective 5-HT_{2A} antagonist, M100907, the non-selective 5-HT_2 receptor antagonist, pirenperone, and the selective HT_{1A} receptor antagonist, WAY-100635, do not block the potentiation of the stimulus effects of PCP by citalopram. In contrast, the selective 5-HT_{2C} receptor antagonist, SDZ SER 082, at

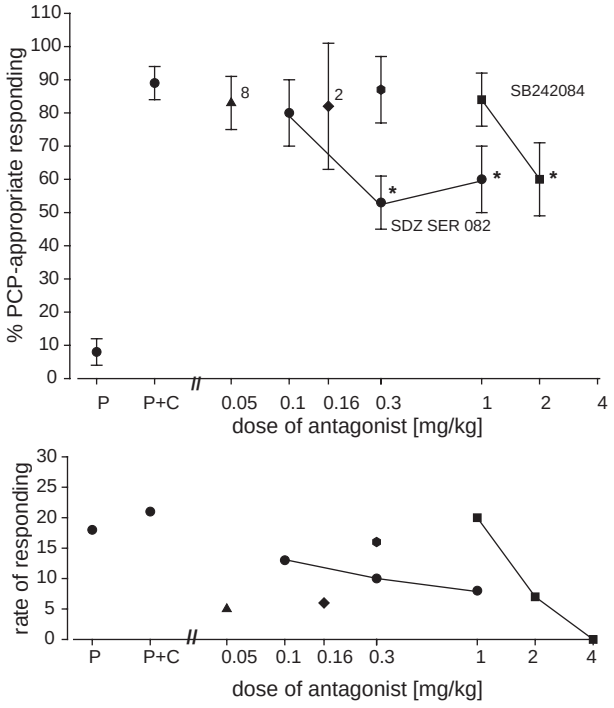

Fig. 2. The effects of selected serotonergic antagonists on the potentiation of the stimulus effects of PCP [1.0 mg/kg; 30 min pretreatment time] following the administration of citalopram [3.0 mg/kg; 90 min pretreatment time]. The point indicated by P on the abscissa is for PCP [1.0 mg/kg] alone. The point indicated by P+C on the abscissa is for the combination of PCP and citalopram. Other points shows the effects of P+C in combination with the 5-HT_{2A} antagonist, M100907 [▲], the 5-HT_2 antagonist, pirenperone [◆], the 5-HT_{1A} antagonist, WAY-100635 [⬢], and the 5-HT_{2C} antagonists, SDZ SER 082 [●] and SB 242084 [■], respectively. All points represent the mean of one determination in each of 10 rats. An asterisk indicates a statistically significant difference between P+C alone and in combination with an antagonist. A numeral adjacent to a point indicates the number of subjects completing the test if other than 10. *Ordinate: Upper panel:* percent PCP-appropriate responding. *Lower panel:* rate expressed as responses per minute. *Abscissa:* dose plotted on a log scale.

doses of 0.3 and 1.0 mg/kg antagonized the interaction of citalopram with PCP [$F(2,9)=22.040$, $P<0.001$; $F(2,9)=20.689$, $P<0.001$, respectively]. Likewise, the selective 5-HT_{2C} receptor antagonist, SB 242084, at a dose of 2.0 mg/kg significantly decreased the interaction between PCP and citalopram [$F(2,9)=30.899$, $P<0.001$]. Subsequent pairwise comparisons revealed significant differences between PCP [1.0 mg/kg] alone, PCP+citalopram, and PCP+citalopram+antagonist thus meeting our criteria for intermediate antagonism. In separate experiments, no statistically significant antagonism of the training dose of PCP was observed in the presence of M100907, pirenperone, WAY-100635, SDZ SER 082, or SB 242084 [data not shown].

3.3. Interaction of the non-selective 5-HT_{2C} receptor agonist, mCPP, with PCP

Based upon the results seen in Fig. 2 with selective antagonists at 5-HT_{2C} receptors, we examined the effect of a non-selective 5-HT_{2C} receptor agonist, *meta*-chlorophenylpiperazine [*m*CPP] (Callahan and Cunningham, 1994; Fiorella et al., 1995b; Pauwels et al., 2003), on the stimulus effects of an intermediate dose of PCP. For doses of PCP and *m*CPP of 1.0 and 0.3 mg/kg, respectively, repeated measures ANOVA revealed a significant increase in PCP-appropriate responding following the combination of *m*CPP and PCP compared with PCP alone [$N=6$; $F(2,5)=11.077$; $P=0.003$].

4. Discussion

We previously provided evidence that stimulus control by LSD and by DOM is enhanced both by SSRIs (Fiorella et al., 1996; Winter et al., 1999a, 2002) and by non-competitive NMDA antagonists including PCP (Winter et al., 2000a). The latter observation of behaviorally significant serotonergic/glutamatergic interactions is extended by the present data (Fig. 1) to include potentiation of PCP-induced stimulus control by the SSRI, citalopram. Because citalopram is selective for the serotonin transporter (Millan et al., 2000), we may rule out a direct role for increased levels of either dopamine or norepinephrine. However, in as much as citalopram would be expected to increase levels of serotonin at all serotonin receptors, the data provided in Fig. 1 do not define the specific receptor or receptors involved. Of the 14 serotonin receptors now recognized (Hoyer et al., 2002), we chose to examine 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} because each may play a role in glutamate release, one of the proposed mechanisms by which not only PCP but also LSD and the phenethylamine hallucinogens exert their behavioral effects (Aghajanian and Marek, 1999, 2000; Winter et al., 2004).

A functionally significant role for activation or antagonism of 5-HT_{1A} receptors in the effects of citalopram as well as non-competitive NMDA antagonists is suggested by several studies. The selective 5-HT_{1A}-selective antagonists WAY-100135 and WAY-100635 antagonize citalopram-induced hypothermia (Oerther and Ahlenius, 2001) and certain of the behavioral effects of the non-competitive NMDA antagonist, dizocilpine [MK-801] (Loscher and Honack, 1993; Wedzony et al., 2000). In addition, dizocilpine increases the number of 5-HT_{1A} receptors in rat brain (Wedzony et al., 1997) and Czyrak et al. (2003) have identified 5-HT_{1A} receptors in the rat cingulate cortex which they believe may regulate glutamate release. However, it is seen in Fig. 2 that the selective 5-HT_{1A} antagonist, WAY-100635, does not alter the potentiating effects of citalopram. The dose chosen, 0.3 mg/kg, was previously shown in our laboratory to fully block the effects of the 5-HT_{1A} agonist, 8-hydroxy-2-dipropylaminotetralin [8-OH-DPAT] (Winter et al., 2000b). The absence of an effect of WAY-100635 on the potentiation of PCP by citalopram is in keeping with our previous observation that WAY-100635 does not alter the effects of citalopram on stimulus control by DOM (Eckler et al., 2004).

It has long been recognized that the stimulus effects of both indoleamine and phenethylamine hallucinogens are mediated by serotonergic receptors (Browne and Ho, 1975; Winter, 1975, 1978) specifically those of the 5-HT_2 subtype (Glennon et al., 1983, 1984; Fiorella et al., 1995a) and that PCP acts via blockade of NMDA receptors (Anis et al., 1983; Zukin and Zukin, 1979; Koek, 1999). In a most provocative hypothesis, Aghajanian and Marek (1999, 2000) proposed on the basis of electrophysiological evidence that release of glutamate represents a final common pathway for hallucinogens whose direct effects are on either NMDA or serotonergic receptors. We have recently provided direct support for this hypothesis by demonstrating that LSD-induced stimulus control is potentiated by a glutamate releaser, LY 341495, and partially antagonized by LY 379268, an $mGlu_{2/3}$ agonist which inhibits glutamate release (Winter et al., 2004). In addition, it has recently been shown using in vivo microdialysis that LSD as well as the phenethylamine hallucinogens, 2,5-dimethoxy-4-methylamphetamine [DOM] and 2,5-dimethoxy-4-iodoamphetamine [DOI], increase extracellular glutamate in rat brain (Scruggs et al., 2003; Muschamp et al., 2004). Moghaddam and Adams (1998) observed similar increases in serotonin levels in rat brain following systemic treatment with PCP. Because LSD-induced release of glutamate is antagonized by the selective 5-HT_{2A} antagonist, M100907 (Muschamp et al., 2004), we tested the hypothesis that citalopram potentiates the stimulus effects of PCP via agonism at 5-HT_{2A} receptors. However, neither M100907 nor pirenperone diminished the effect of citalopram on stimulus control by PCP (Fig. 2) It should be noted that the doses of pirenperone [0.16 mg/kg] and M100907 [0.05 mg/kg] used in the present study have previously been found to antagonize completely the stimulus effects of LSD in Fischer 344 rats (Winter and Rabin, 1988; Winter et al., 2004). A puzzling aspect of the interaction between the combination of PCP and citalopram and the 5-HT_2 receptor antagonists, M100907 and pirenperone, is the rate decreasing effect seen in Fig. 2; indeed, following pirenperone, only 2 of 10 subjects completed the interaction tests. Previously we observed similar rate-suppressing effects of pirenperone in combination with agents presumed to act as agonists at 5-HT_{1A} receptors (Winter and Rabin, 1988). Although we are unaware of definitive behavioral data with respect to the selectivity of pirenperone for 5-HT_2 receptor subtypes, in vitro second messenger studies suggest a 250-fold higher affinity for the 5-HT_{2A} subtype as compared with the 5-HT_{2C} subtype (Hoyer et al., 1994).

Abundant evidence suggests that serotonin plays a significant role in glutamate release or glutamatergic functions and most studies implicate the 5-HT_{2A} receptor (Arvanov et al., 1999; Meller et al., 2002; Regina et al., 2004). However, the fact that there is a highly significant correlation between agonist activity at 5-HT_{2A} and 5-HT_{2C} receptors (Nichols, 2004) has made difficult an assessment of the part played by the latter receptor. Nonetheless, glutamate release by serotonergic agonists such as LSD or DOI is fully blocked by M100907 (Scruggs et al., 2003; Muschamp et al., 2004) and, in those studies in which selective 5-HT_{2C} antagonists have been employed, negative results have been obtained (Marcoli et al., 2001; Martin-Ruiz et al., 2001; Dawson et al., 2002; Pei et al., 2004). Despite these caveats, a prominent role for 5-HT_{2C} receptors in the actions of citalopram is suggested by the studies of Millan and his colleagues (Millan et al., 1999; Dekeyne et al., 2001) in which citalopram was trained as a discriminative stimulus in the rat. Citalopram generalized to the selective 5-HT_{2C} agonist, Ro 60-0175, and was blocked by the selective 5-HT_{2C} antagonist, SB 242084 (Kennett et al., 1997). Furthermore, previous work in our laboratory provided evidence that sensitization to the stimulus effects of LSD following serotonin depletion in the rat is accompanied by upregulation of the 5-HT_{2C} receptor (Fiorella et al., 1995c). In addition, we previously observed that SB 242084 significantly but incompletely blocks augmentation of the stimulus effects of DOM by citalopram (Eckler et al., 2004). For these reasons, we examined SB 242084 (Kennett et al., 1997) and SDZ SER 082 (Nozulak et al., 1995) in combination with citalopram and PCP (Fig. 2). The results obtained strongly suggest that, indeed, the 5-HT_{2C} receptor is a significant factor in the effects of citalopram on DOM. However, the fact that antagonism by both drugs was intermediate in nature, i.e., statistically significant but less than complete, leaves open the possibility that other factors are involved. A further test of the importance of actions at the 5-HT_{2C} receptor would be a demonstration that agonists at 5-HT_{2C} receptors potentiate the stimulus effects of PCP. To that end, we examined the interaction with PCP of the 5-$HT_{2C/2B}$ agonist, *m*CPP and significant potentiation of an intermediate dose of PCP was observed. Unfortunately, the more selective 5-HT_{2C} receptor agonist, Ro 60-0175, used by Millan et al. (1999) to characterize the stimulus effects of citalopram was not available to us due to institutional constraints. Nonetheless, the observed effects of *m*CPP on PCP-induced stimulus control together with the observation that potentiation of PCP by citalopram is significantly antagonized by SB 242084 and by SDZ SER 082 support the hypothesis that citalopram acts to potentiate stimulus control by DOM via a 5-HT_{2C}-mediated mechanism.

It should be noted that a racemic mixture of citalopram was employed in the present studies. In future investigations it would be well to examine the respective contributions of the [*R*]- and [*S*]-isomers of citalopram. Recent evidence suggests that the [*S*]-isomer [escitalopram] is the more active of the two (Hyttel et al., 1992) and that, indeed, [*R*]-citalopram may antagonize certain of the effects of escitalopram (Mork et al., 2003; Sanchez, 2003; Fish et al., 2004). In our previous studies of the potentiation of the phenethylamine hallucinogen, DOM, by citalopram (Eckler et al., 2002), pharmacokinetic factors were ruled out by the measurement of DOM levels in the brain. Although citalopram is believed to interact minimally with cyto-

chrome *P*450 [CYP] enzymes (for reviews, see Brosen and Naranjo, 2001; Spina et al., 2003), the fact that PCP is metabolized by CYP enzymes (Laurenzana and Owens, 1997) makes plausible a pharmacokinetic contribution to the present behavioral interaction between PCP and citalopram. This hypothesis was not tested in the present investigation.

The present data add to the body of evidence which indicates that functionally significant interactions occur between glutamatergic and serotonergic systems (Aghajanian and Marek, 2000; Carlsson et al., 2001; Winter et al., 2000a, 2004). These interactions may provide important clues as to the mechanisms of action of multiple classes of hallucinogenic drugs as well to reconcile and to integrate current hypotheses as to the etiology of psychotic disorders. We suggest that drug-induced stimulus control, a behavioral technique which over the past three decades has contributed to our understanding of a variety of psychoactive drugs may provide fresh insight into serotonergic/glutamatergic interactions.

Acknowledgments

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