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M100907, a Serotonin 5-HT_{2A} Receptor Antagonist and Putative Antipsychotic, Blocks Dizocilpine-Induced Prepulse Inhibition Deficits in Sprague–Dawley and Wistar Rats

Geoffrey B. Varty, Ph.D., Vaishali P. Bakshi, Ph.D., and Mark A. Geyer, Ph.D.

In a recent study using Wistar rats, the serotonergic 5-HT_2 receptor antagonists ketanserin and risperidone reduced the disruptive effects of the noncompetitive N-methyl-D-aspartate (NMDA) antagonist dizocilpine on prepulse inhibition (PPI), suggesting that there is an interaction between serotonin and glutamate in the modulation of PPI. In contrast, studies using the noncompetitive NMDA antagonist phencyclidine (PCP) in Sprague–Dawley rats found no effect with 5-HT_2 antagonists. To test the hypothesis that strain differences might explain the discrepancy in these findings, risperidone was tested for its ability to reduce the PPI-disruptive effects of dizocilpine in Wistar and Sprague–Dawley rats. Furthermore, to determine which serotonergic receptor subtype may mediate this effect, the 5-HT_{2A} receptor antagonist M100907 (formerly MDL 100,907) and the 5-HT_{2C} receptor antagonist SDZ SER 082 were tested against dizocilpine. Recent studies have found that the PPI-disruptive effects of PCP are reduced by the α_1 adrenergic receptor antagonist prazosin. Furthermore, the α_1 receptor agonist cirazoline disrupts PPI. As risperidone and M100907 have affinity at the α_1 receptor, a final study examined whether M100907 would block the effects of cirazoline on PPI. Risperidone partially, but nonsignificantly, reduced the effects of dizocilpine in Wistar rats, although this effect was smaller than previously reported. Consistent with previous studies, risperidone did not alter the effects of dizocilpine in Sprague–Dawley rats. Most importantly, M100907 pretreatment fully blocked the effect of dizocilpine in both strains; whereas SDZ SER 082 had no effect. M100907 had no influence on PPI by itself and did not reduce the effects of cirazoline on PPI. These studies confirm the suggestion that serotonin and glutamate interact in modulating PPI and indicate that the 5-HT_{2A} receptor subtype mediates this interaction. Furthermore, this interaction occurs in at least two rat strains.
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KEY WORDS: *Rat; Strain; Startle; Prepulse inhibition; Serotonin; Glutamate; Dizocilpine; M100907, Schizophrenia*

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Prepulse inhibition (PPI) refers to the reduction in startle magnitude produced by a low intensity, nonstartling prepulse that immediately precedes the startling stimulus (Hoffman and Ison 1980). PPI models a form of preattentional sensorimotor gating and is reduced in psychiatric disorders characterized by deficient sensorimotor gating, such as schizophrenia (Braff et al. 1978, 1992; Grillon et al. 1992; Bolino et al. 1994).

Deficits in PPI have been produced in rats by the systemic administration of dopamine and serotonin agonists or noncompetitive NMDA antagonists (see Geyer et al. 1990 for review). The effects of dopamine agonists

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are blocked by dopamine D_2 receptor antagonists, including both typical and atypical antipsychotics (Mansbach et al. 1988; Swerdlow et al. 1994). Similarly, the corresponding receptor subtype antagonists block the effects of serotonin agonists (Sipes and Geyer 1994). In particular, the PPI-disruptive effects of 5-HT_2 agonists are blocked specifically by the selective 5-HT_{2A} antagonist M100907 (Sipes and Geyer 1995). The PPI-disruptive effects of noncompetitive NMDA antagonists, such as phencyclidine (PCP), dizocilpine (or MK-801), and ketamine, are not blocked by dopamine receptor antagonists (Geyer et al. 1990; Keith et al. 1991; Hoffman et al. 1993; Bakshi et al. 1994; Varty and Higgins 1995a), but are reduced by multireceptor antagonist antipsychotics, such as clozapine, risperidone, quetiapine (Seroquel), and olanzapine (Bakshi et al. 1994; Bakshi and Geyer 1995; Varty and Higgins 1995a; Swerdlow et al. 1996). Accordingly, several recent studies have examined the particular receptors that might contribute to the ability of these atypical antipsychotics to reduce the PPI-disruptive effects of NMDA antagonists. In brief, selective antagonists at dopaminergic, muscarinic, GABAergic, and α_2 adrenergic receptors have failed to reduce these effects of noncompetitive NMDA antagonists (Keith et al. 1991; Bakshi et al. 1994; Varty and Higgins 1995a).

The possible contributions of serotonergic 5-HT_{2A} or 5-HT_{2C} receptors to the interaction between clozapine-like drugs and noncompetitive NMDA antagonists have remained unclear, however. Recent studies demonstrated that the disruptive effect of dizocilpine on PPI was reduced by pretreatment with either the serotonin 5-HT_2 receptor antagonist ketanserin or the mixed serotonin 5-HT_2/dopamine D_2 receptor antagonist risperidone (Varty and Higgins 1995a,b). These novel findings indicate that there is an interaction between serotonin and glutamate in the modulation of PPI. This interaction is particularly interesting in view of the reports that both serotonin and glutamate are implicated in the neurobiology of schizophrenia (see Halberstadt 1995; Iqbal and van Praag 1995; Tamminga et al. 1995). In contrast, studies by another group demonstrated that similar 5-HT_2 antagonists failed to prevent the PPI-disruptive effect of PCP (Bakshi et al. 1994, Swerdlow et al. 1996). There were several experimental differences between the studies that may have contributed to the discrepancy in findings, including the strain of rat used (Wistar vs. Sprague–Dawley rats), the time of testing in relation to the animals' light/dark cycle, the design of the PPI testing sessions, the baseline-matching technique, the NMDA antagonists used (dizocilpine vs. PCP), and the drug doses tested. We hypothesized that one likely explanation for the different findings with 5-HT_2 antagonists might be the difference in rat strain. Previous studies have shown that PPI and its pharmacological manipulation differ between strains of rat. For example, the PPI disruptive effects of both PCP and the dopamine agonist apomorphine differ between Wistar, Sprague–Dawley, and Lister Hooded rat strains (Rigdon 1990, Varty and Higgins 1994); furthermore, strain differences are apparent when examining the blockade of an apomorphine-induced PPI disruption by the atypical antipsychotic clozapine (Swerdlow et al. 1998).

To evaluate our hypothesis, risperidone was tested for its ability to block the PPI-disruptive effects of dizocilpine in groups of Wistar and Sprague–Dawley rats, using the same drug doses as those in the Varty and Higgins (1995b) study. Furthermore, as risperidone has its highest serotonergic affinity at the 5-HT_{2A} and 5-HT_{2C} receptor subtypes (Schotte et al. 1996), if, indeed, there is an interaction with glutamate, we hypothesized that at least one of these receptors is likely mediating this effect. To test this hypothesis, we examined the effects of the selective 5-HT_{2A} antagonist and putative antipsychotic M100907 (Kehne et al. 1996) and the selective 5-HT_{2C} antagonist SDZ SER 082 (Nozulak et al. 1993, 1995) on the PPI-disruptive effect of dizocilpine. Finally, recent studies have shown that the PPI-disruptive effect of PCP is reduced by the α_1 adrenergic receptor antagonist prazosin (Bakshi and Geyer 1997); furthermore, treatment with the α_1 adrenergic receptor agonist cirazoline disrupts PPI (Carasso et al. 1998). Risperidone and M100907 have moderate affinity at the α_1 receptor at the doses tested. Therefore, a final study examined whether α_1 receptors contribute to the observed interaction between M100907 and dizocilpine by testing M100907 against the PPI-disruptive effect of cirazoline in Sprague–Dawley rats.

MATERIALS AND METHODS

Subjects

Male Sprague–Dawley and Wistar rats (both from Harlan, San Diego, CA) weighing 300 to 350 g were used. Animals were housed two per cage in a room controlled for constant temperature and humidity, with food and water available *ad libitum*. Animals were maintained on a 12 h reversed light/dark cycle (lights off 7:30 h, lights on 19:30 h) and all testing occurred between 9:00 to 17:00 h. Animals were handled at least 2 days before testing to reduce any subsequent handling stress. All animals experiments were carried out in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publications No. 80-23, revised 1978).

Drugs

Dizocilpine maleate (RBI, USA), cirazoline hydrochloride (RBI, USA), and SDZ SER 082 (Sandoz, Switzerland) were dissolved in 0.9% saline solution. Risperidone (Janssen, Belgium) was dissolved in a small

amount of 1M hydrochloric acid (approximately 50 μl), made up to the correct volume with saline and buffered to pH 5-6 with 1M sodium hydroxide. M100907 (Hoechst Marion Roussel, Inc., USA) was dissolved in a warm 15% Tween80 (Sigma, USA) solution. All drugs were administered subcutaneously except for cirazoline and risperidone, which were administered by the intraperitoneal route. All doses are expressed as free base.

Apparatus

Four startle chambers were used for measuring the startle response (SR-LAB, San Diego Instruments, San Diego, CA). Each chamber consisted of a *Plexiglas* cylinder (9-cm diameter) mounted on a frame, housed within a ventilated chamber (39 × 38 × 58 cm). Sudden movements within the cylinder were detected by a piezoelectric accelerometer attached below the cylinder. A loudspeaker (Radio Shack Supertweeter) mounted 24 cm above the cylinder provided the broadband background noise and acoustic stimuli. An internal 15-W light bulb provided light prepulse stimuli. Presentations of the acoustic and light stimuli were controlled by the SR-LAB software and interface system, which also rectified, digitized (0-4095), and recorded responses from the accelerometer. As described previously (Mansbach et al. 1988), sound levels [dB(A) scale] and accelerometer sensitivities within each chamber were calibrated regularly and found to remain constant over the test period.

Experimental Protocol

Animals were pretreated with an injection of vehicle or an antagonist 30 min [1.0 mg/kg SDZ SER 082 or 1.0 mg/kg M100907] or 60 min [1.0 mg/kg risperidone] before testing. Immediately prior to testing, the same animals were treated with an injection of saline, 0.15 mg/kg dizocilpine, or 0.75 mg/kg cirazoline and placed into the startle chambers. All four drug combinations were represented in each test run [n = 8–11 per group] and were balanced across the four startle chambers.

The experimental session consisted of a 5 min acclimatization period to a 65 dB background noise (continuous throughout the session), followed by 20 min acoustic and 5 min light PPI test sessions. At least 4 days prior to any drug testing, animals were pre-exposed to the chambers and the testing session. The purpose of the pre-exposure was to acclimatize the animals to the testing chambers and startle/prepulse stimuli, and to baseline-match the groups for subsequent testing (groups were matched for equivalent mean startle magnitude and % PPI, see below for definitions). During the acoustic PPI session, five trial types were presented: a 40 ms, 120 dB startle pulse (P120); and four prepulse + pulse combinations: A 71 dB or 77 dB prepulse (6 and 12 dB above background, respectively; both 20 ms in duration) followed 100 ms later by a P120 stimulus, or a 71 dB or 77 dB prepulse followed 300 ms later by a P120 stimulus. Each trial type was presented in pseudorandom order (24 presentations of P120 trial, 12 presentations of each prepulse + pulse combination) with an average intertrial interval (ITI) of 15 s. In addition, six P120 trials were presented at both the beginning (to familiarize the animals to the stimulus) and the end of the acoustic test session (to separate the acoustic and light PPI sessions). These trials were not included in calculations of startle magnitude.

Data and Statistical Analysis

Mean startle magnitude for each trial type presentation, the dependent measure, was determined by averaging 100 1-ms readings taken from the beginning of the main startle stimulus (P120). The level of PPI (% PPI) was determined according to the formula [1 - (startle magnitude on prepulse + pulse trials/startle magnitude on pulse (P120) trials) × 100], so that a 0% value indicated no difference between the responses to prepulse + pulse trials and P120 trials (i.e., no PPI).

Mean startle magnitude from the P120 trials was analyzed using a three-way analysis of variance (ANOVA), with strain, pretreatment, and treatment as between-subjects factors. Percentage acoustic PPI data were analyzed using a five-way ANOVA with prepulse intensity and ISI as within-subjects factors, and strain, pretreatment, and treatment as between-subjects factors (cirazoline study used the same ANOVAs without strain as a factor). If there were no significant interactions with intensity and/or ISI, the mean percentage acoustic PPI was calculated by collapsing the acoustic PPI over the appropriate prepulse combinations. Mean percentage PPI data were analyzed using a three-way ANOVA with strain, pretreatment, and treatment as between-subjects factors. If there was an interaction with one of the parameters, a four-way ANOVA was used with the noninteraction parameter (intensity or ISI) as the within-subjects factor and strain, pretreatment, and treatment as the between-subjects factors. For the sake of brevity, F-values are only described following significant effects. Furthermore, main effects of prepulse intensity and ISI will not be described, because these effects were significant in every study. *Post hoc* comparisons were made using Tukey's t-tests with an acceptance level of $p < .05$.

RESULTS

Risperidone vs. Dizocilpine

There were significant main effects of strain [$F(1,56) = 7.1$, $p = .01$], risperidone pretreatment [$F(1,56) = 32$, $p < .01$], and dizocilpine treatment [$F(1,56) = 13$, $p < .01$] on

startle magnitude, but no interactions. In both strains, risperidone seemed to reduce startle responding; whereas, dizocilpine increased startle magnitude in Sprague–Dawley rats (Figure 1, Tables 1 and 2). In the analysis of PPI, there were significant main effects of risperidone pretreatment [$F(1,56) = 18$, $p < .01$] and dizocilpine treatment [$F(1,56) = 73$, $p < .01$]. There were no interactions and no main effect of strain. Dizocilpine disrupted PPI in both strains and, based on our *a priori* hypothesis that risperidone would reduce the PPI-disruptive effects of dizocilpine, risperidone seemed to partially reduce this effect in both strains, but more so in the Wistar rats (see Figure 1, Tables 1 and 2). However, there was no interaction between risperidone and dizocilpine, and there was a main effect of risperidone to increase PPI by itself. Therefore, we cannot conclusively state that risperidone pretreatment blocked the disruptive effect of dizocilpine on PPI in these studies.

M100907 vs. Dizocilpine

There were significant main effects of strain [$F(1,55) = 10$, $p < .01$] and dizocilpine treatment [$F(1,55) = 8.4$, $p < .01$] on startle magnitude, but no effect of M100907 pretreatment and no interactions. Dizocilpine increased startle magnitude in Sprague–Dawley rats but had no effect in Wistars (Figure 2, Tables 1 and 2). The ANOVA of PPI revealed significant main effects of M100907 pretreatment [$F(1,55) = 8.8$, $p < .01$] and dizocilpine treatment [$F(1,55) = 79$, $p < .01$], but no main effect of strain. Most importantly, there was a significant interaction between M100907 pretreatment and dizocilpine on PPI [$F(1,55) = 13$, $p < .01$]. Dizocilpine significantly reduced PPI and M100907 blocked this effect in both strains (see Figure 2, Tables 1 and 2). There were, however, strain by prepulse intensity [$F(1,55) = 6.3$, $p < .05$], and strain by ISI [$F(1,55) = 6.6$, $p = .01$] interactions (strains differed in their sensitive to intensity and ISI manipulations; that is, Wistars seemed more sensitive; compare PPI data in Tables 1 and 2), and a strain by dizocilpine treatment interaction [$F(1,55) = 6.5$, $p = .01$] (dizocilpine seemed to produce larger disruptions in the Sprague–Dawley strain). Most importantly, in both strains, pretreatment with M100907 had no effect on basal PPI and reduced the PPI disruptions produced by dizocilpine treatment.

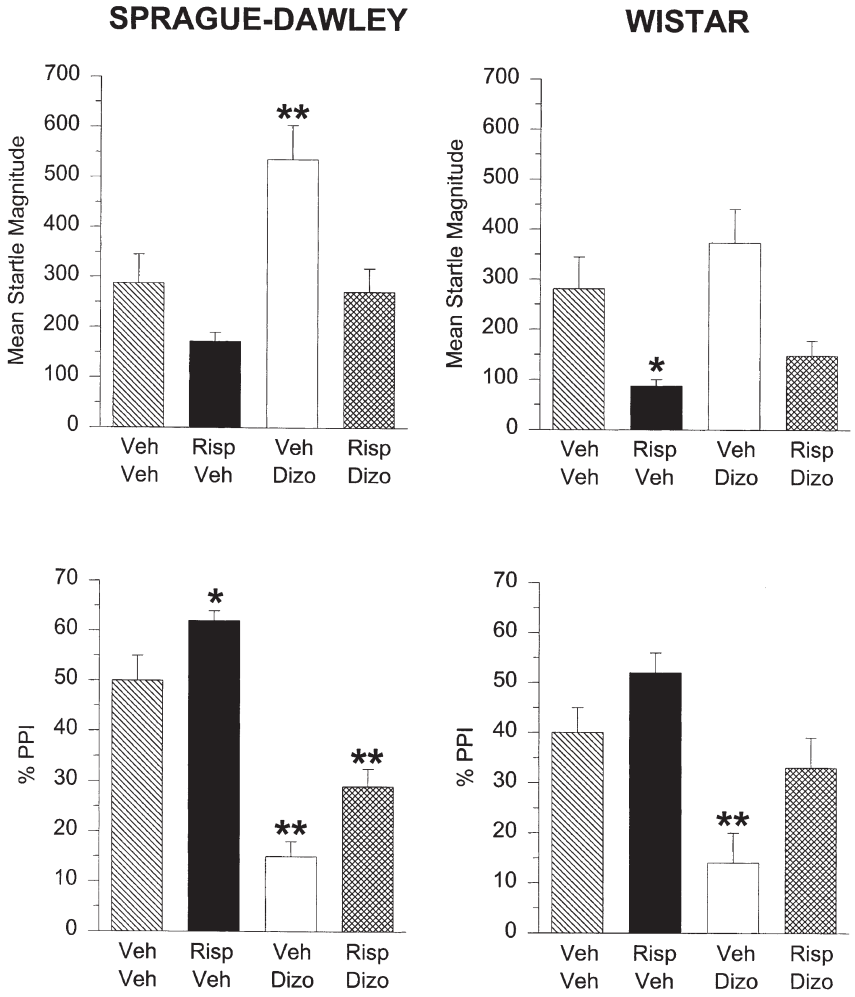

Figure 1. Effects of risperidone (1.0 mg/kg) and dizocilpine (0.15 mg/kg) on startle magnitude and prepulse inhibition (PPI) in Sprague–Dawley and Wistar rats. Top figures represent mean startle magnitude in both strains, and the bottom figures represent mean percentage PPI (collapsed across prepulse + pulse combinations). Veh = vehicle; Risp = risperidone; Dizo = dizocilpine. Values are mean ± SEM per group. * $p < .05$; ** $p < .01$ vs. veh/veh.

Table 1. Risperidone (1.0 mg/kg), M100907 (1.0 mg/kg), and SDZ SER 082 (1.0 mg/kg) Pretreatment on the Effects of Dizocilpine (0.15 mg/kg) on Percentage Prepulse Inhibition (PPI) (Four Prepulse + Pulse Combinations) in Sprague–Dawley rats

	Prepulse Combination			
	71 dB, 100 ms	**77 dB, 100 ms**	**71 dB, 300 ms**	**77 dB, 300 ms**
Risperidone				
Vehicle/vehicle	50 ± 6	63 ± 7	34 ± 7	53 ± 7
Risperidone/vehicle	58 ± 3	72 ± 2	50 ± 3	69 ± 2
Vehicle/dizocilpine	14 ± 3**	24 ± 5**	−5 ± 6**	26 ± 4**
Risperidone/dizocilpine	25 ± 5**	42 ± 4*††	15 ± 4*††	35 ± 9
M100907				
Vehicle/vehicle	55 ± 6	73 ± 5	50 ± 5	64 ± 5
M100907/vehicle	60 ± 4	70 ± 6	48 ± 7	64 ± 6
Vehicle/dizocilpine	−7 ± 7**	11 ± 7**	0.2 ± 6**	5 ± 8**
M100907/dizocilpine	30 ± 8*††	43 ± 8*††	25 ± 6*††	42 ± 7*††
SDZ SER 082				
Vehicle/vehicle	31 ± 4	55 ± 3	25 ± 5	48 ± 5
SDZ SER 082/vehicle	37 ± 8	56 ± 7	35 ± 6	55 ± 7
Vehicle/dizocilpine	19 ± 6**	34 ± 8**	16 ± 5	29 ± 6**
SDZ SER 082/dizocilpine	5 ± 7**	32 ± 6**	−4 ± 6**	28 ± 6**

Values are mean ± SEM.
*$p < .05$; **$p < .01$ vs. vehicle/vehicle; †$p < .05$; ††$p < .01$ vs. vehicle/dizocilpine.

SDZ SER 082 vs. Dizocilpine

There were significant main effects of strain [F(1,72) = 4.0, $p = .05$] and dizocilpine treatment [F(1,72) = 21, $p < .01$] on startle magnitude, but no effect of SDZ SER 082 pretreatment and no interactions. Dizocilpine increased startle magnitude in both strains (Figure 3, Tables 1 and 2). Analysis of PPI data indicated that there was a significant main effect of dizocilpine treatment [F(1,72) = 42, $p < .01$], but no effects of SDZ SER 082 pretreatment [F(1,72) < 1, NS] or strain [F(1,72) < 1, NS]. Dizocilpine disrupted PPI in both strains. Furthermore, pretreatment with SDZ SER 082 had no effect on basal PPI and did not block the PPI disruptions following dizocilpine treatment (see Figure 3 for percentage PPI data at 77 dB, 300 ms combination, and Tables 1 and 2 for percentage PPI data at all four prepulse + pulse combinations).

Table 2. Risperidone (1.0 mg/kg), M100907 (1.0 mg/kg), and SDZ SER 082 (1.0 mg/kg) Pretreatment on the Effects of Dizocilpine (0.15 mg/kg) on Percentage Prepulse Inhibition (PPI) (Four Prepulse + Pulse Combinations) in Wistar Rats

	Prepulse Combination			
	71 dB, 100 ms	**77 dB, 100 ms**	**71 dB, 300 ms**	**77 dB, 300 ms**
Risperidone				
Vehicle/vehicle	41 ± 6	56 ± 7	24 ± 5	41 ± 7
Risperidone/vehicle	41 ± 5	66 ± 3	41 ± 6*	58 ± 5*
Vehicle/dizocilpine	10 ± 6**	25 ± 8**	4 ± 6*	19 ± 8*
Risperidone/dizocilpine	26 ± 8	45 ± 7	19 ± 6	44 ± 6†
M100907				
Vehicle/vehicle	44 ± 6	64 ± 6	33 ± 4	50 ± 6
M100907/vehicle	39 ± 5	62 ± 5	26 ± 5	47 ± 7
Vehicle/dizocilpine	11 ± 4**	29 ± 8**	4 ± 5**	16 ± 9**
M100907/dizocilpine	23 ± 5*	50 ± 5†	10 ± 6**	36 ± 6†
SDZ SER 082				
Vehicle/vehicle	41 ± 4	62 ± 4	25 ± 4	56 ± 3
SDZ SER 082/vehicle	29 ± 7	59 ± 4	28 ± 6	48 ± 7
Vehicle/dizocilpine	14 ± 6*	31 ± 8**	−5 ± 7**	29 ± 6**
SDZ SER 082/dizocilpine	5 ± 8**	35 ± 7**	3 ± 8	31 ± 6*

Values are mean ± SEM.
*$p < .05$; **$p < .01$ vs. vehicle/vehicle; †$p < .05$ vs. vehicle/dizocilpine.

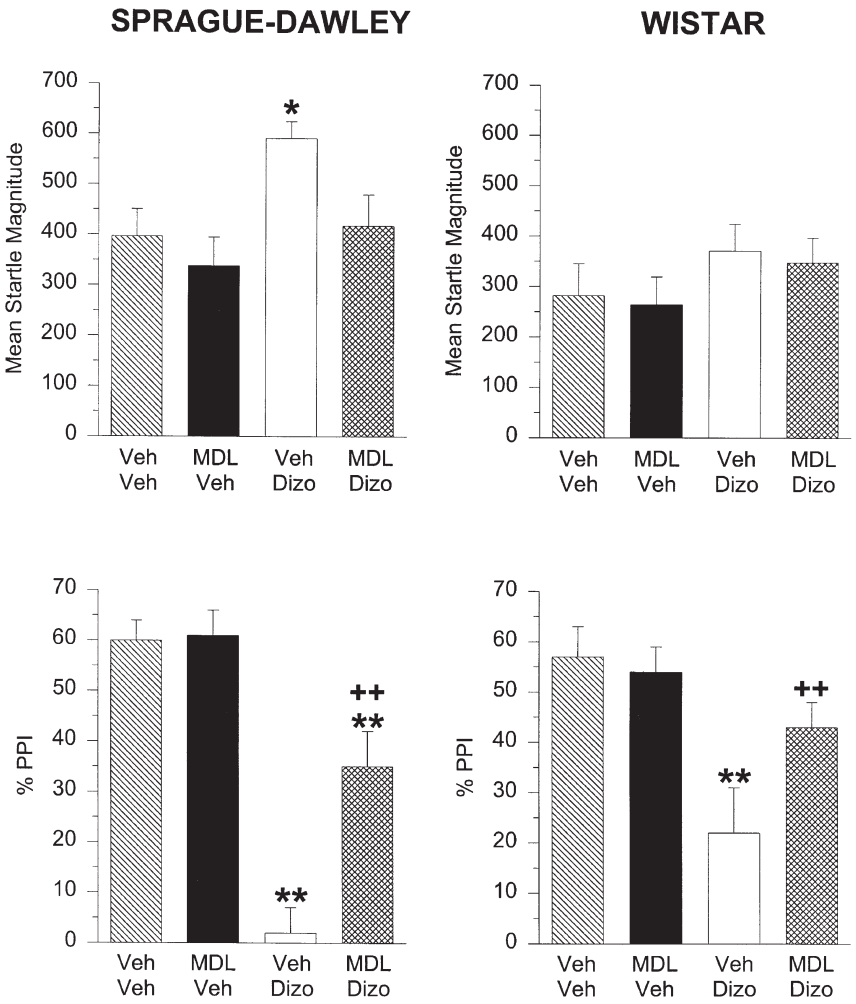

Figure 2. Effects of M100907 (1.0 mg/kg) and dizocilpine (0.15 mg/kg) on startle magnitude and prepulse inhibition (PPI) in Sprague–Dawley and Wistar rats. Top figures represent mean startle magnitude in both strains, and the bottom figures represent mean percentage PPI (collapsed across prepulse + pulse combinations). Veh = vehicle; MDL = M100907; Dizo = dizocilpine. Values are mean ± SEM per group. * $p < .05$; ** $p < .01$ vs. veh/veh; $^{++}$ $p < .01$ vs. veh/dizo.

There were however, strain by prepulse intensity [$F(1,72) = 5.2$, $p < .05$], prepulse intensity by pretreatment by treatment [$F(1,72) = 6.4$, $p = .01$], and prepulse intensity by ISI by pretreatment by treatment [$F(1,72) = 6.5$, $p = .01$] interactions. These interactions were caused largely by a potentiation by SDZ SER 082 of the dizocilpine-induced disruption of PPI (i.e., PPI was reduced further) at the 71 dB prepulse combination, particularly in the Sprague–Dawley strain (see Tables 1 and 2). A similar trend has been seen in previous studies of SDZ SER 082 (Bakshi et al. 1994). It should be noted that this apparent effect of SDZ SER 082 in the present study was restricted to the 71 dB condition and was not confirmed in a retest of the same Sprague–Dawley rats in which we balanced the previous treatments (data not shown).

M100907 vs. Cirazoline in Sprague–Dawley Rats

There was a significant effect of cirazoline treatment [$F(1,38) = 11.3$, $p < .01$] to increase startle magnitude, with no effect of M100907 pretreatment and no interaction (see Figure 4). With regard to PPI, there was a significant effect of cirazoline treatment [$F(1,38) = 60$, $p < .01$], but no effect of M100907 pretreatment. In addition, there were interactions between treatment and ISI [$F(1,38) = 18$, $p < .01$] and treatment, ISI, and intensity [$F(1,38) = 7$, $p = .01$]. Examining the PPI data over the prepulse conditions, it is clear that M100907 had no effect on PPI by itself or against the cirazoline-induced disruption in Sprague–Dawley rats (see Figure 4 for percentage PPI data at 77 dB, 300 ms ISI combination, or Table 3 for all PPI data).

DISCUSSION

The major finding of the present studies was that the selective 5-HT_{2A} antagonist and putative antipsychotic M100907 robustly blocked the effects of dizocilpine on PPI in both Sprague–Dawley and Wistar rats. By contrast, the 5-HT_{2C} antagonist SDZ SER 082 completely failed to reduce the effects of dizocilpine in either strain

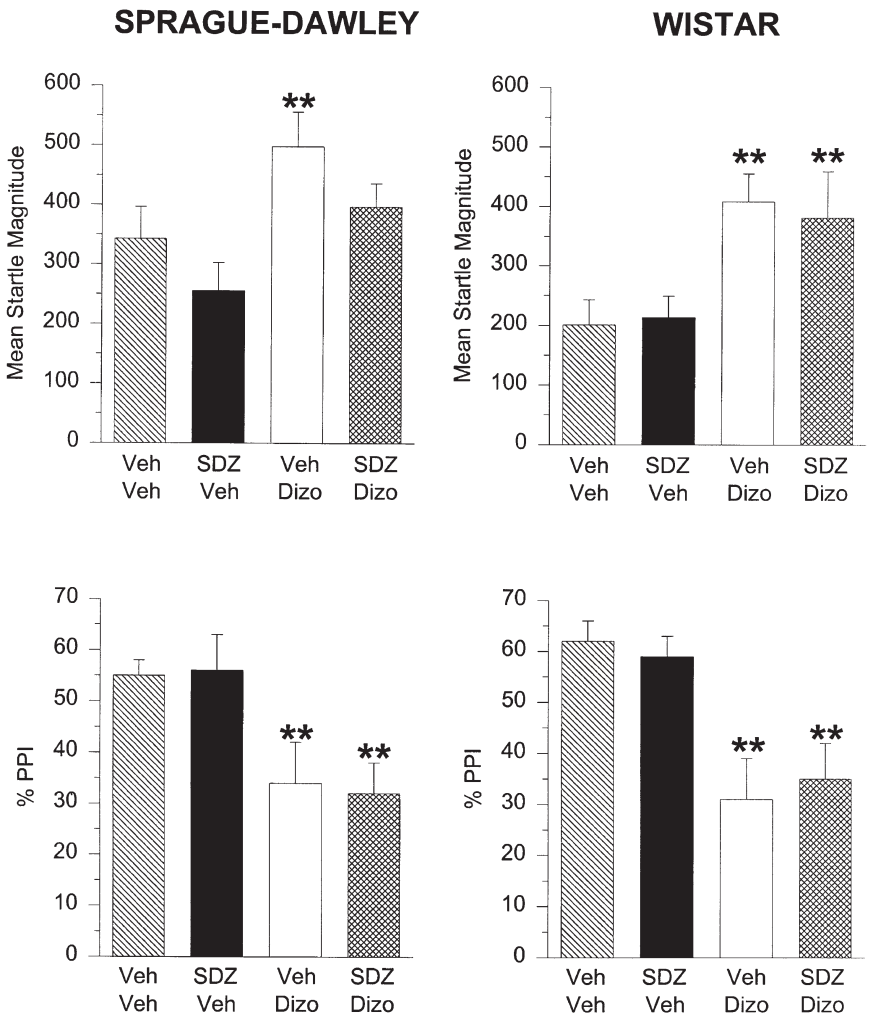

Figure 3. Effects of SDZ SER 082 (1.0 mg/kg) and dizocilpine (0.15 mg/kg) on startle magnitude and prepulse inhibition (PPI) in Sprague–Dawley and Wistar rats. Top figures represent mean startle magnitude in both strains, and the bottom figures represent percentage PPI (at 77 dB, 100 ms prepulse + pulse combination). Veh = vehicle; SDZ= SDZ SER 082; Dizo = dizocilpine. Values are mean ± SEM per group. ** $p <$ .01 vs. veh/veh.

of rats. Furthermore, the mixed 5-HT_2/D_2 receptor antagonist risperidone, although it reduced startle reactivity and increased PPI by itself, may have marginally reduced the effect of dizocilpine in Wistar rats. Together with previous reports (Varty and Higgins 1995a,b), these findings indicate that 5-HT_{2A} receptor actions may contribute to the efficacy of clozapine and other multireceptor antagonist antipsychotics in blocking the PPI-disruptive effects of NMDA antagonists.

A previous report using Wistar rats had suggested that there was a potentially interesting interaction between serotonin and glutamate in the modulation of PPI (Varty and Higgins 1995b). In studies using Sprague–Dawley rats, the same interaction was not evident (Bakshi et al. 1994; Swerdlow et al. 1996). Hence, the present study examined whether this disparity is further evidence of rat strain differences in the mediation and modulation of PPI. In the studies of risperidone and dizocilpine, significant main effects in the absence of pretreatment by treatment interactions indicated that risperidone increased PPI and dizocilpine decreased PPI. Thus, although the effect of dizocilpine on PPI seemed to be reduced by risperidone in Wistar, but not in Sprague–Dawley rats (Figure 1), we cannot definitively conclude that risperidone blocked the PPI-disruptive effects of dizocilpine, even in Wistar rats. Therefore, we did not replicate convincingly the finding of Varty and Higgins (1995b) in Wistar rats, but did confirm the finding of Swerdlow et al. (1996) in Sprague–Dawley rats. Despite using the same drug doses, pretreatment times, and routes of administration, the partial reduction of the dizocilpine effect by risperidone in Wistar rats was smaller than that seen in the previous study (Varty and Higgins 1995b). This difference may be attributable to other inconsistencies. For example, the animals in this study were run during the animal's dark cycle, whereas in the previous study, animals were run during the light cycle, the PPI sessions were slightly different, and the Wistar rats were obtained from different vendors in different countries. It is conceivable that future studies using the optimal conditions for each strain of rat, or a range of risperidone doses, could detect a significant, cleaner effect of risperidone to reduce dizocilpine-induced disruptions of

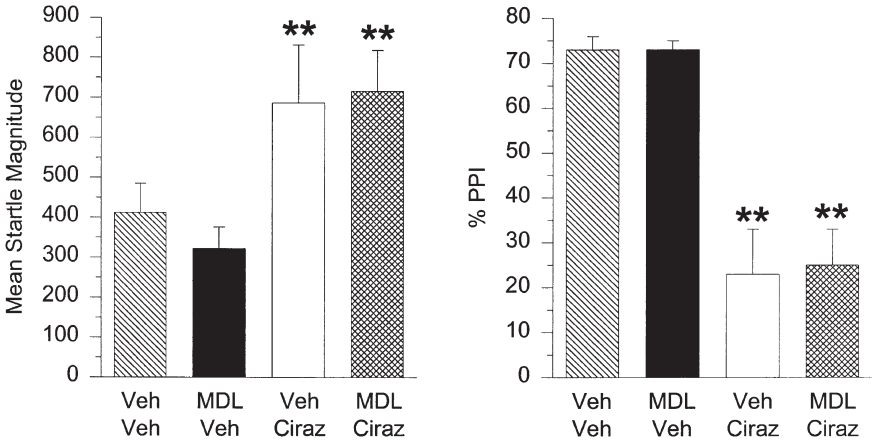

Figure 4. Effects of M100907 (1.0 mg/kg) and cirazoline (0.75 mg/kg) on mean startle magnitude and prepulse inhibition (percentage PPI at 77 dB, 300 ms prepulse + pulse combination) in Sprague–Dawley rats. Veh = vehicle; MDL = M100907; Ciraz = cirazoline. Values are mean ± SEM per group. ** $p < .01$ vs. veh/veh.

PPI (see also Swerdlow et al. 1996). The effects of risperidone to reduce startle magnitude and increase basal PPI are probably attributable to the high dopamine D_2 receptor affinity of the compound (Schotte et al. 1996). Similar effects are exhibited when D_2 antagonists, such as haloperidol and raclopride, are tested in the PPI paradigm (Swerdlow and Geyer 1993; Varty and Higgins 1995a). The high D_2 receptor affinity of risperidone does not account for its potential ability to reduce the dizocilpine effect, because neither haloperidol nor raclopride reduce the effects of dizocilpine or PCP on PPI (Geyer et al. 1990; Keith et al. 1991; Hoffman et al. 1993; Bakshi et al. 1994; Varty and Higgins 1995a).

The results of the studies with the 5-HT_{2A} antagonist M100907 were much clearer. M100907 blocked the PPI-disruptive effect of dizocilpine in both strains without affecting startle responding or PPI when given alone. The blockade by M100907 was larger in magnitude than risperidone, and in the Wistar rats there was a complete reversal of the dizocilpine effect at the 77 dB prepulse intensity. Furthermore, the reversal of dizocilpine by M100907 in Wistar rats was independent of the concomitant effects of risperidone on startle magnitude and PPI that complicated interpretation of the risperidone data. The experiments using a behaviorally active dose of the selective 5-HT_{2C} antagonist SDZ SER 082 (Nozulak et al. 1993), did not reveal any similar effect of the 5-HT_{2C} antagonist on the PPI-disruptive effects of dizocilpine, suggesting negligible involvement of this receptor subtype. Indeed, if SDZ SER 082 had any effect, it was to potentiate rather than to reduce the effects of dizocilpine, consistent with a similar trend observed in studies of the interaction of SDZ SER 082 and PCP in the PPI model (Bakshi et al. 1994). If such an effect were confirmed, it could explain the apparent differences between the actions of the selective 5-HT_{2A} antagonist M100907 seen in the present studies and the actions of less selective 5-HT_2 antagonists, such as risperidone and ketanserin, in previous studies (Bakshi et al. 1994; Swerdlow et al. 1996). In any event, the finding that M100907 blocks the PPI-disruptive effects of dizocilpine provides further evidence for an interaction between serotonin 5-HT_2 and glutamate systems in the modulation of PPI, in support of the findings of Varty and Higgins (1995b). The blockade by M100907 of the effects of dizocilpine was seen in both Sprague–Dawley and Wistar rats, indicating that the interaction is generalizable across strains. A variety of previous studies have demonstrated serotonin/glutamate interactions in the control of locomotor behavior, mostly based on nonse-

Table 3. M100907 (1.0 mg/kg) Pretreatment on the Effects of Cirazoline (0.75 mg/kg) on Percentage Prepulse Inhibition (PPI) (Four Prepulse + Pulse Combinations) in Sprague–Dawley Rats

	Prepulse Combination			
	71 dB, 100 ms	**77 dB, 100 ms**	**71 dB, 300 ms**	**77 dB, 300 ms**
Vehicle/vehicle	60 ± 5	76 ± 3	43 ± 5	73 ± 3
M100907/vehicle	58 ± 6	75 ± 3	52 ± 4	73 ± 2
Vehicle/cirazoline	−7 ± 17**	22 ± 14**	8 ± 10**	23 ± 10**
M100907/cirazoline	−15 ± 8**	8 ± 10**	9 ± 6**	25 ± 8**

Values are mean ± SEM.
**$p < .01$ vs. vehicle/vehicle.

lective 5-HT_2 antagonists (Kitaichi et al. 1994; Varty and Higgins 1995b; Gleason and Shannon 1997) and some based on interactions of PCP with both 5-HT_2 agonists and antagonists (Krebs-Thomson et al. 1998). Furthermore, recent studies of the effects of M100907 in both rats and mice demonstrate that these serotonin/glutamate interactions are likely attributable specifically to the 5-HT_{2A} receptor subtype (Maurel-Remy et al. 1995; Martin et al. 1997). Similarly, the lack of effect with SDZ SER 082 coupled with the effects of M100907 in the present studies suggests that the 5-HT_{2A} receptor subtype mediates the serotonin/glutamate interaction with respect to the modulation of PPI as well. Of final note, a recent *in vitro* study demonstrated that M100907 facilitates NMDA receptor transmission in rat medial prefrontal cortex (Arvanov and Wang 1998). Cortical glutamate hypofunction may underlie some of the symptomatology of schizophrenia, particularly the negative symptoms. The authors suggest that any efficacy of M100907 against negative symptoms may be attributable to this enhancement of cortical glutamate transmission. As the authors have noted similar results with clozapine and a range of 5-HT_2 antagonists, including ketanserin, ritanserin, and metergolline, they suggest that the facilitatory effect of M100907 on glutamate transmission is possibly mediated via 5-HT_{2A} receptors. Thus, the study by Arvanov supports our findings in which we demonstrated a potential serotonin/glutamate interaction in an *in vivo* model of a schizophrenic-like behavioral deficit that is sensitive to novel, atypical antipsychotic drugs.

A recent series of studies by Bakshi and Geyer (1997) demonstrated that the effects of PCP on PPI are reduced by pretreatment with the α_1 adrenergic receptor antagonist prazosin. Clozapine and olanzapine also reduce the effects of PCP on PPI (Bakshi et al. 1994; Bakshi and Geyer 1995) and both have high affinity at α_1 and 5-HT_{2A} receptors (Schotte et al. 1996). The ability of these atypical antipsychotics to reduce the effects of the PCP may reflect an indirect involvement of α_1 and/or 5-HT_{2A} receptors in the PPI-disruptive effects of PCP (see Bakshi and Geyer 1997). In addition, a recent study found that the α_1 adrenergic receptor agonist cirazoline disrupts PPI, evidence for an α_1 adrenergic component to the regulation of PPI (Carasso et al. 1998). Risperidone has 15 times greater affinity for the 5-HT_{2A} receptor than for the α_1 adrenergic receptor, but nonetheless, still possesses relatively high affinity for the α_1 receptor (Schotte et al. 1996). Therefore, it is conceivable that the ability of risperidone to reduce dizocilpine in the study by Varty and Higgins (1995b) may represent a similar interaction with α_1 adrenergic receptors. M100907, however, has 150 times greater affinity for the 5-HT_{2A} receptor than for the α_1 adrenergic receptor. Furthermore, unlike clozapine and risperidone, M100907 is ineffective in a behavioral model of α_1 receptor antagonism (Kehne et al. 1996). In a related study, ziprasidone, a novel 5-HT_{2A}/D_2 receptor antagonist with low α_1 receptor affinity, was found to reduce the effects of the noncompetitive NMDA antagonist ketamine on PPI (Brooks and Mansbach 1997). Thus, it seems that the effect of M100907 to block dizocilpine is not attributable to effects at α_1 adrenergic receptors. To corroborate this conclusion, we examined whether the α_1 adrenergic component of M100907 was important by testing M100907 against a PPI-disruptive dose of cirazoline (Carasso et al. 1998). Pretreatment with M100907, at the same dose that blocked the effects of dizocilpine, did not block the PPI-disruptive effect of cirazoline. Therefore, the α_1 adrenergic component of the 5-HT_{2A} antagonist M100907 is not responsible for the blockade of dizocilpine-induced disruptions of PPI.

Determining the serotonergic receptor subtype mediating the serotonin/glutamate interaction was a secondary aim of our studies; therefore, we only tested one dose of each antagonist. Selective and potent doses of each antagonists were determined from published studies of SDZ SER 082 and M100907 (see Nozulak et al. 1993, 1995; Kehne et al. 1996); however, only full dose–response studies for each antagonist would ensure that our findings are, in fact, reflecting actions at the specific receptor subtype. In addition, the lack of a clear effect with risperidone in these studies, as compared to previous studies (see Varty and Higgins 1995a,b), may simply reflect a difference in drug potency between laboratories; therefore, it is conceivable that studies examining higher doses of risperidone may produce results similar to those seen with M100907. Nevertheless, there is clearly an interaction between glutamate and serotonergic 5-HT_2 antagonists, and more importantly, putative serotonergic antipsychotics, in the modulation of PPI.

As a final note, we did see some evidence for rat strain differences in these studies. First, Sprague–Dawley rats seem to have a higher level of startle reactivity than Wistars, as indicated by main effects of strain. Interestingly, this startle reactivity difference is opposite to that seen in a previous study in the United Kingdom using the same rat strains, but from different vendors (Varty and Higgins 1994). This difference in startle reactivity between laboratories may be attributable to other factors. For example, the difference in vendors supplying the animals or the period of the light cycle in which animals were tested; that is, animals tested during the light phase may demonstrate changes in startle reactivity, as compared to animals tested during the dark phase. Only studies addressing these points directly can answer this question. Second, dizocilpine robustly increased startle reactivity in the Sprague–Dawley strain; whereas, this effect was either absent in the Wistars, or smaller in magnitude; however, dizocilpine clearly disrupted PPI in both strains despite different effects on startle reactivity. Third, the antagonistic effect of M100907 on dizocilpine differed between the strains; that is, the

effect was more robust in the Sprague–Dawley strain. Finally, SDZ SER 082 seemed to reduce the startle-potentiating effects of dizocilpine in the Sprague–Dawley rat but had no such effect in the Wistar strain. Again, despite the strain differences in startle effects of SDZ SER 082, SDZ SER 082 was ineffective at blocking the effects of dizocilpine on PPI in both strains. Collectively, these findings support the importance of addressing strain differences, and potential vendor differences in behavioral paradigms. Clearly, rat strains not only differ in their normal behavior, but they also demonstrate marked differences in drug-induced changes in behavior, both of which are important considerations when interpreting behavioral data. In these studies however, M100907 reversed the effect of dizocilpine on PPI in the two strains, suggesting that the interaction between serotonin and glutamate may be evident across a spectrum of rat strains. Only comprehensive studies in additional, commonly used strains (e.g. Lister Hooded, Fischer F344, Lewis, Long Evans) can empirically answer this question.

In summary, we failed to replicate convincingly the finding that risperidone can reduce the disruptive effect of dizocilpine on PPI in Wistar rats (Varty and Higgins 1995b), while corroborating reports that nonselective 5-HT_2 antagonists are ineffective in this regard in Sprague–Dawley rats (Bakshi et al. 1994; Swerdlow et al. 1996). Most importantly, the present studies demonstrated a blockade of the effects of dizocilpine using the selective 5-HT_{2A} antagonist M100907 in both Wistar and Sprague–Dawley rats, at a dose that did not block the PPI-disruptive effects of the α_1 adrenergic agonist, cirazoline. Thus, antagonist actions at 5-HT_{2A} receptors may contribute importantly to the ability of multireceptor antagonist antipsychotics, such as clozapine, to reduce the PPI-disruptive effects of noncompetitive NMDA antagonists. Furthermore, the blockade of a dizocilpine-induced disruption of PPI by M100907 supports a serotonin/glutamate interaction in the modulation of PPI, previously suggested by Varty and Higgins (1995b). These findings, along with the lack of effect of the 5-HT_{2C} antagonist SDZ SER 082 indicate that this interaction is likely mediated via actions at the 5-HT_{2A} receptor subtype.

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