Phencyclidine-Induced Deficits in Prepulse Inhibition of Startle are Blocked by Prazosin, an Alpha-1 Noradrenergic Antagonist

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Abstract
Prepulse inhibition (PPI) is a form of plasticity of the startle response in which presentation of a weak stimulus immediately before an intense starting stimulus reduces the resultant startle response. Deficits in PPI, an operational measure of sensorimotor gating, are observed in schizophrenia patients and can be modeled in rats by the psychotogen phencyclidine (PCP). PCP-induced deficits in PPI in rats are resistant to dopamine and serotonin antagonists but can be antagonized by antipsychotics such as clozapine, olanzapine and Seroquel. These latter antipsychotics have antagonistic actions at several receptors, including alpha-1 and alpha-2 adrenergic, M1 muscarinic and gamma-aminobutyric acid (GABA)-A receptors. Although the direct actions of PCP are thought to be mediated by noncompetitive antagonism of N-methyl-D-aspartate sites, PCP thereby indirectly activates multiple neurotransmitter systems, including those affected by the aforementioned antipsychotics. The present studies examined the possibility that an antagonist action at a particular receptor subtype might be responsible for the interaction between PCP and the clozapine-like antipsychotics by testing whether a selective antagonist at alpha-1, alpha-2, M1 or GABA-A receptors would prevent the PCP-induced deficit in PPI in rats. Animals were pretreated with either the alpha-1 antagonist prazosin (0, 0.5, 1.0 or 2.5 mg/kg), the alpha-2 antagonist RX821002 (0, 0.2 or 0.4 mg/kg), the M1 muscarinic antagonist pirenzepine (0, 10 or 30 mg/kg) or the GABA-A antagonist pitrazepin (0, 1.0 or 3.0 mg/kg) and then treated with either saline or PCP (1.5 mg/kg). Because prazosin was effective in blocking the effects of PCP, an additional experiment tested the possibility that prazosin (0, 1.0 or 2.5 mg/kg) would block the PPI deficits produced by the dopamine agonist apomorphine (0 or 0.5 mg/kg). After drug administration, animals were tested in startle chambers. PCP was found to decrease PPI. Prazosin (1.0 and 2.5 mg/kg) blocked this deficit in two separate experiments but did not increase base-line PPI levels. The effects on PPI were dissociable from changes in startle reactivity. Furthermore, prazosin did not antagonize apomorphine-induced disruptions of PPI, which suggests that the antagonism of the PCP effect was not simply due to a generalized improvement of deficient PPI. The antagonists for alpha-2, for M1 and for GABA-A receptors had no effect on base-line PPI or on PCP-induced disruptions in PPI. These findings indicate that the PPI-disruptive effect of PCP may be mediated in part by alpha-1 adrenergic receptors and that antagonism of alpha-1 receptors may play a major role in mediating the blockade of PCP-induced deficits in PPI by certain antipsychotics.

PCP is a dissociative anesthetic, the clinical use of which was discontinued after burgeoning reports of its psychotomimetic profile (see Balster, 1987). It has since been demonstrated that PCP is a powerful psychotogen that in healthy humans elicits a wide range of symptoms that resemble both the positive and the negative symptomatology observed in schizophrenia, and acutely exacerbates existing behavioral and cognitive impairments in schizophrenia patients (Javitt, 1987; Allen and Young, 1978; Luisada, 1978; Snyder, 1988). Ketamine, another dissociative anesthetic that is closely related to PCP, has also been shown to produce psychotic symptoms and cognitive disintegration in healthy volunteers (Krystal et al., 1994).

The deficits in central inhibitory mechanisms that likely contribute to psychotic symptoms in schizophrenia have been quantified using manipulations of the startle response. The startle response is an involuntary response to sudden intense stimuli that can be inhibited or gated by presentation of a weak prepulse immediately before the startling stimulus (Hoffman and Ison, 1980; Ison and Hoffman, 1983; Braff and Geyer, 1990). This ubiquitous phenomenon of PPI is one form...
of plasticity of the startle response and provides an operational measure of sensorimotor gating, a central inhibitory or filtering mechanism that is deficient in schizophrenia and schizotypal patients (Braff et al., 1978; 1992; Bolino et al., 1994; Cadenhead et al., 1993; Grillon et al., 1992). In results consistent with its psychotomimetic profile, PCP has been found in rats to disrupt PPI (Geyer et al., 1990; Mansbach and Geyer, 1989), mimicking the PPI deficits that are observed in the aforementioned psychiatric populations. Moreover, ketamine disrupts PPI in both rats and humans at subanesthetic doses, which is further evidence of the ability of psychotomimetic compounds of this class to impair sensorimotor gating (Karper et al. 1994; Krystal et al., 1994; Mansbach and Geyer, 1991).

Although the impairment in sensorimotor gating produced by PCP is well documented (Bakshi et al., 1994; Bakshi and Geyer, 1995; Swerdlow et al., 1996; Johansson et al., 1994b; Mansbach and Geyer, 1989; Wiley, 1994), the neuropharmacological mechanisms by which this effect is exerted remain unclear. PCP is an open channel blocker of the NMDA ionophore complex, acting as a noncompetitive antagonist of the NMDA receptor. As a consequence, PCP has myriad indirect facilitatory effects on monoaminergic and cholinergic pathways in the brain. For example, PCP increases DA release, augments ACh efflux, decreases acetylcholinesterase activity and inhibits 5-HT and NE reuptake (Bowyer et al., 1984; Hondo et al., 1994; Hutson and Hogg, 1996; Hori et al., 1996; Johnson and Hillman, 1982; Rogers and Lemaire, 1991; Smith et al., 1977). PCP has also been reported to increase extracellular levels of GABA (Lilranks et al., 1994). Antipsychotics such as clozapine have been shown to partially block deficits in PPI produced by PCP and other noncompetitive NMDA antagonists (Bakshi et al., 1994; Bakshi and Geyer, 1995; Swerdlow et al., 1996), in contrast to dopamine antagonists, which do not block PPI disruptions induced by non-competitive NMDA antagonists (Geyer et al., 1990; Keith et al., 1991; Hoffman et al., 1993; Swerdlow et al., 1996). Because compounds such as clozapine have not been shown to possess appreciable affinity for the NMDA receptor itself, it is likely that the source of interaction between PCP and clozapine-like antipsychotics is at a systems level through the various neurotransmitter systems that are indirectly activated by PCP (Moore et al., 1993; Coward, 1992; Saller and Salama, 1993). Clozapine has antagonistic properties at receptors within all of the neurotransmitter systems that are indirectly activated by PCP, which indicates that there are multiple possible sites of interaction between the effects of these two compounds. Thus, identification of a receptor-selective antagonist that blocks the PPI-disruptive effects of PCP would indicate one source of interaction between the clozapine-like antipsychotics and PCP. That is, whereas the primary mechanism of PCP action is presumed to involve the NMDA receptor, clozapine-like antipsychotics may act by blocking a secondary effect of PCP distal to the relevant NMDA receptors.

Previous studies of selective antagonists for D1, D2, and 5-HT2 receptors have shown these compounds to be ineffective in blocking the deficit in PPI produced by PCP (Bakshi et al., 1994), which suggests that these receptors are probably not responsible for the interaction between clozapine and PCP. In addition to its actions on DA and 5-HT receptors, clozapine also has a high affinity for alpha-1, alpha-2 and muscarinic M1 receptors (Coward, 1992). Furthermore, studies of the GABA ion channel indicate that clozapine functions as a GABA-A antagonist in vitro (Squires and Saedrup, 1993). It has yet to be determined, however, whether antagonists at these additional receptors will prevent PCP-induced disruptions of PPI. The purpose of the present investigation was to determine whether alpha-1, alpha-2, M1 or GABA-A receptors might be involved in the interaction between clozapine-like antipsychotics and PCP. Specifically, we tested whether selective antagonists for alpha-1, alpha-2, M1 or GABA-A receptors (given individually) would block PCP-induced deficits in PPI.

## Materials and Methods

### Animals

A total of 338 male Sprague-Dawley rats (Harlan Laboratories, San Diego, CA), weighing 300 to 400 g, were used in the present studies. Animals were housed in pairs in clear plastic cages located inside a temperature- and humidity-controlled animal colony and were maintained on a reversed day/night cycle (lights on from 7:00 P.M. to 7:00 A.M.). Food (Harlan Teklad, Madison, WI) and water were available continuously except during behavioral testing, which occurred between 9:00 A.M. and 5:00 P.M. Upon arrival in the colony, all animals were handled gently by the experimenter in order to minimize stress during behavioral testing. Animal facilities were AAALAC-approved; protocols were in accordance with the “Guiding Principles in the Care and Use of Animals” (provided by the American Physiological Society) and the guidelines of the National Institutes of Health.

### Drugs

The following drugs were used: PCP hydrochloride (1.5 mg/kg); apomorphine hydrochloride (0.5 mg/kg), both from Sigma Chemical Co. (St. Louis, MO); prazosin hydrochloride (0.5, 1.0 or 2.5 mg/kg); RX21002 hydrochloride (0.2 or 0.4 mg/kg); pirenzepine dihydrochloride (10 or 30 mg/kg), all from (Research Biochemicals International Natick, MA) and pitrazepin (1.0 or 3.0 mg/kg) (Novartis Pharmaceuticals, Basel, Switzerland). PCP, RX21002, pirenzepine and pitrazepin were dissolved in isotonic saline. Apomorphine was dissolved in saline with 0.1% ascorbic acid. Prazosin was dissolved in either a vehicle of distilled water and dimethyl sulfoxide (5%) or a vehicle solution of saline (50%), propylene glycol (40%) and ethanol (10%). All doses were calculated as the salt. Injection volume was 1 ml/kg for all drugs except pitrazepin, which was administered in a volume of 2 ml/kg.

### Apparatus

Behavioral testing. One week after arrival, all rats underwent a brief startle session in order to create matched treatment groups. In this session and the subsequent test session, the background noise (65 dB) was presented alone for 5 min and then continued throughout the remainder of the session. A total of 20 trials were presented in a pseudo-random order: 17 presentations of a 40-msec 120-dB broadband burst and 3 trials in which a 77-dB burst preceded the 120-dB burst by 100 msec. Treatment groups were established by using the mean startle response to the 120-dB PULSE-ALONE trial...
so that all groups had comparable base-line startle reactivity. One to two days after the base-line session, drug testing began. The test session utilized in all of the experiments contained five different trial types and had a total duration of 20 min: a PULSE-ALONE trial in which a 40-msec, 120-dB broadband burst was presented; three PREPULSE + PULSE trials in which 20-msec noises that were 3, 6 or 12 dB above the background noise were presented 100 msec before the onset of the 120-dB pulse and a NO STIMULUS trial, which included only the background noise. All trial types were presented several times in a pseudo-random order for a total of 52 trials (20 PULSE-ALONE trials and 8 each of the remaining trial types). In addition, 4 PULSE-ALONE trials, which were not included in the calculation of PPI values, were presented at the beginning of the test session to achieve a relatively stable level of startle reactivity for the remainder of the session (based on the observation that the most rapid habituation of the startle reflex occurs within the first few presentations of the startling stimulus (Geyer et al., 1990)). An average of 15 sec (ranging from 9 to 21 sec) separated consecutive trials.

**Experimental design.** Six experiments were conducted using separate groups of animals. In all experiments with PCP, the dose utilized was 1.5 mg/kg (s.c., 10 min before entry into startle chambers). This PCP regimen was chosen because it has been found previously to produce a robust and reliable deficit in PPI that can be antagonized by atypical antipsychotics (Bakshi and Geyer, 1995; Swerdlow et al., 1991). In experiment 1, we examined the possibility that the alpha-1 antagonist prazosin (Hornung et al., 1979) would block PCP-induced deficits in PPI. Rats were pretreated i.p. with either vehicle or 1.0 or 2.5 mg/kg prazosin and 20 min later were given either saline or PCP. Because both prazosin doses were found to antagonize the effects of 1.5 mg/kg PCP, we carried out a separate experiment in order to evaluate a lower dose of prazosin. Thus, in experiment 2, either vehicle or 0.5 mg/kg prazosin was administered using the same protocol as in experiment 1.

Experiment 3 tested the hypothesis that prazosin antagonized PPI disruptions in PCP-treated animals by producing a generalized improvement in deficient PPI. Thus the possibility that prazosin would prevent deficits in PPI produced by a DA agonist (apomorphine) was examined. Animals were pretreated i.p. with either vehicle or 1.0 or 2.5 mg/kg prazosin and 20 min later were given either vehicle or apomorphine (0.5 mg/kg s.c.). This dose of apomorphine was selected because it has been shown repeatedly to produce a disruption of PPI that is antagonized by clozapine and other antipsychotics (Mansbach et al., 1988; Swerdlow et al., 1991). Rats were placed into startle chambers immediately after the second injection.

In order to assess the receptor subtype selectivity of the prazosin effect, we examined in experiment 4 the possibility that an alpha-2 antagonist, RX821002 (Langin et al., 1989), would reverse the PCP-induced PPI deficit. Either saline or 0.2 or 0.4 mg/kg RX821002 was injected i.p. 20 min before PCP administration.

Experiment 5 tested the possibility that a M1 muscarinic antagonist, pirenzepine (Luthin and Wolfe, 1984), would prevent the PCP-induced deficit in PPI. Rats were pretreated i.p. with either vehicle or 10 or 30 mg/kg pirenzepine and 20 min later were given either saline or PCP.

In the final experiment, we evaluated the effects of a GABA-A antagonist, pitrazepin (Gahwiler et al., 1984), on PCP-induced PPI deficits. The rationale for this study was that clozapine, which antagonizes the PCP-induced PPI deficit (Bakshi et al., 1994), has in vitro effects on the GABA chloride channel that are identical to the effects of selective GABA-A antagonists (Squires and Saedrup, 1993). Thus experiment 6 sought to determine whether the antagonism by clozapine of the PCP effects on PPI occurred via an indirect interaction through the GABA-A system. Piztrazepin (vehicle or 1.0 or 3.0 mg/kg i.p.) was administered 20 min before either saline or PCP.

**Data analysis.** The startle response to the 120-dB burst was recorded for each PULSE-ALONE and PREPULSE + PULSE trial. Two measures were calculated from these data for each animal. First, the amount of PPI was calculated as a percentage score for each PREPULSE + PULSE trial type: % PPI = 100 - ([startle response for PREPULSE + PULSE trial]/startle response for PULSE-ALONE trial) × 100. Second, startle magnitude was calculated as the average response to all of the PULSE-ALONE trials. All PPI data were analyzed with three-factor analysis of variance (ANOVA) with pretreatment and treatment as between-subject factors and trial type (prepulse intensity) as a repeated measure. Startle magnitude data were analyzed with two-factor (pretreatment and treatment) ANOVA. Post-hoc analyses were carried out using Tukey’s test. P level was set at .05.

**Results**

**Effect of prazosin on PCP-induced behavior.** Experiment 1 yielded several important results that are illustrated in figure 1. First, administration of PCP resulted in an abolition of PPI, evidenced by a statistically significant main effect of treatment [F(1,36) = 50.88, P < .001]. Subsequent analyses indicated that PCP disrupted PPI at all three prepulse intensities (P < .01). A main effect of pretreatment was also observed [F(2,36) = 5.80, P < .007], which is consistent with the small augmentation of PPI produced by prazosin at all three prepulse intensities (fig. 1). Post-hoc analyses, however, failed to reveal a statistically significant difference between either dose of prazosin and the vehicle condition in saline-treated animals at any prepulse intensity. Interestingly, a nearly significant prepulse intensity × pretreatment × treatment interaction was obtained through ANOVA [F(4,72) = 2.36, P < .061]. As depicted in figure 1, prazosin pretreatment increased PPI in PCP-treated animals by as much as 300%, nearly returning PPI in these animals to control values. Given the magnitude of this effect, the a priori hypothesis for this experiment, and the significant main effects of treatment and pretreatment, we conducted post-hoc analyses. The results of these subsequent analyses confirmed the blockade of the PCP-induced deficit by prazosin. Animals that received the lower dose of prazosin in conjunction with
PCP exhibited significantly higher levels of PPI at both the 6-dB (P < .01) and the 12-dB (P < .05) prepulse intensities than animals that received only PCP. Similarly, animals that were pretreated with the 2.5 mg/kg dose before PCP administration also had greater PPI than their vehicle-pretreated controls (P < .05, 12-dB prepulse intensity). Thus prazosin was found significantly to antagonize the deficit in PPI produced by PCP.

Experiment 2 sought to extend the dose-response analysis of this effect by testing the possibility that a lower dose of prazosin might also antagonize the PCP-induced deficit. Figure 2 depicts the effects of 0.5 mg/kg of prazosin on basal and PCP-disrupted PPI. As in experiment 1, a large main effect of PCP treatment was observed [F(1,28) = 63.24, P < .001] and confirmed with post-hoc analyses, which revealed a significant disruption in PPI at all three prepulse intensities (P < .01). Unlike the previous experiment, however, neither a significant main effect of prazosin pretreatment [F(1,28) = 0.17, N.S.] nor a pretreatment × treatment interaction [F(1,28) = 1.02, N.S.] was seen, which indicated that this low dose of prazosin did not improve PPI in either saline-treated or PCP-treated animals.

In contrast to PPI, startle magnitude was not affected by either prazosin or PCP in experiment 1, as evidenced by there being no pretreatment main effect, treatment main effect or pretreatment × treatment interaction (table 1). These results indicate that the antagonism of PCP-induced deficits in PPI by prazosin is independent of effects on startle reactivity. In experiment 2, a significant main effect of pretreatment was observed [F(1,28) = 6.45, P < .017]. Subsequent analyses revealed that prazosin-pretreated animals had lower startle reactivity than vehicle-treated controls (P < .05). PCP did not affect startle reactivity in experiment 2.

It should be noted that the subjects for experiment 1 had previously been tested for locomotor activity in response to administration of a 5-HT1A agonist, 8-OH-DPAT. Although all of the treatment groups in the present PPI study (experiment 1) contained roughly equal numbers of animals from each of the previous treatment groups (either vehicle or 3, 6 or 12 μg/kg 8-OH-DPAT), a separate study of prazosin effects on PCP-induced disruption of PPI was conducted using drug-naïve and experimentally naïve rats. In this study, ANOVA revealed a significant pretreatment × treatment interaction [F(1,32) = 4.81, P < .036]. Post-hoc comparisons indicated that PCP significantly disrupted PPI and that prazosin (1.0 mg/kg) significantly antagonized this disruption, a result that replicates the findings of experiment 1. To avoid redundancy, these data are not presented here.

Effect of prazosin on apomorphine-induced behavior. Experiment 3 assessed the effects of prazosin on apomorphine-induced deficits in PPI. The purpose of this experiment was to test the hypothesis that the antagonism of the PCP effect by prazosin might simply be attributed to a generalized improvement of deficient PPI. Analysis of variance revealed a significant main effect of apomorphine treatment [F(1,36) = 98.92, P < .001], which confirmed that apomorphine disrupted PPI, as illustrated in figure 3. This effect was observed at the 3- (P < .01), 6- (P < .05) and 12- (P < .01) dB prepulse intensities. Thus, as was observed with PCP, apomorphine produced a nearly complete loss of PPI in vehicle-pretreated animals. In contrast, no main effect of prazosin pretreatment was observed [F(2,36) = 0.34, N.S.]. Furthermore, in contrast to the two PCP studies, no significant interactions between pretreatment and treatment were seen [F(2,36) = 0.55, N.S.], which indicates that prazosin failed to antagonize the apomorphine-induced deficit in PPI.

Table 1 summarizes the effects of prazosin on apomorphine-induced changes in startle magnitude. A significant main effect of pretreatment was observed [F(2,36) = 6.27, P < .005]. This effect was probably due to the strong interaction between pretreatment and treatment [F(2,36) = 10.59, P < .001]. Post-hoc analyses revealed that animals in the vehicle/apomorphine group had higher startle magnitude than those in the vehicle/vehicle group (P < .01). Prazosin did not affect startle magnitude in vehicle-treated animals, but it did significantly decrease this measure in apomorphine-treated rats. Animals that received either dose of prazosin and then apomorphine had lower startle magnitude than animals that received only apomorphine (P < .01). Thus prazosin antagonized the increase in startle reactivity produced by apomorphine, in contrast to its lack of effect on apomorphine-induced deficits in PPI.

In parallel to the effects on startle magnitude, prazosin also prevented the increase in motor activity, as indexed by the score from the NO STIMULUS trials (Mansbach et al., 1988), that was produced by apomorphine (table 2). Analysis of variance indicated a main effect of treatment [F(1,36) = 25.10, P < .001]. Post-hoc analyses revealed that apomorphine-treated animals had higher NO STIMULUS scores than vehicle-treated controls (P < .01). In contrast, no main effect of pretreatment was observed. Animals that received either dose of prazosin before apomorphine had lower scores than those that received only apomorphine (roughly a 50% reduction), which indicates that prazosin also antagonized increases in overall activity that were produced by apomorphine. It should be noted that no effects on this measure were observed in any of the other studies and that therefore those data are not presented. Finally, a separate apomorphine study was conducted with a longer (30-min) pretest injection interval for prazosin. This experiment yielded the same pat-
Effects of pretreatment with PCP on prepulse inhibition (PPI). Animals that received PCP had significantly lower levels of PPI than animals that received saline. Neither a main effect of pretreatment nor an interaction between factors was indicated. The probable source of this interaction was the statistically insignificant tendency of PCP to augment PPI in saline-treated animals but further decrease PPI in PCP-treated rats (fig. 5). Thus the M₁ muscarinic antagonist also did not block the deficit in PPI produced by PCP.

No effects were observed on startle magnitude (table 1).

Effect of GABA-A antagonist on PCP-induced behavior. Figure 6 shows the results of the final experiment. In experiment 6, PCP was again found to disrupt PI, as indicated by a main effect of treatment [F(1,36) = 40.26, P < .001]. No main effect of pretreatment was observed [F(2,36) = 0.59, N.S.], but a prepulse intensity \( \times \) pretreatment \( \times \) treatment interaction was found [F(4,72) = 2.74, P < .036]. The probable source of this interaction was the statistically insignificant tendency of pirenzepine to augment PPI in saline-treated animals but further decrease PPI in PCP-treated rats (fig. 5). Thus the M₁ muscarinic antagonist also did not block the deficit in PPI produced by PCP.

Knowledge of the variance of the startle magnitude data revealed a main effect of PCP treatment [F(1,42) = 9.72, P < .004]. Although post-hoc analyses failed to reveal significant differences between any of the treatment groups, it is evident from examination of the mean values that PCP tended to increase startle magnitude, irrespective of pirenzepin pretreatment (table 1).

Discussion

Several important results were obtained from the present studies. First, in accordance with many previous reports, PCP was found repeatedly to disrupt PI (Bakshi and Geyer, 1995; Bakshi et al., 1994; Mansbach and Geyer, 1989; Swerdlov et al., 1996; Wiley, 1994; Varty and Higgins, 1994; on startle magnitude was indicated by ANOVA. Although subsequent analyses did not find any of the treatment groups to be significantly different from each other, it is apparent that PCP tended to increase startle magnitude and that this trend was not altered by RX821002 (table 1).

Effect of M₁ muscarinic antagonist on PCP-induced behavior. The effects of pirenzepine on PCP-induced deficits in PPI are depicted in figure 5. As in the previous studies, a significant main effect of treatment was revealed by ANOVA [F(1,36) = 40.26, P < .001]. No main effect of pretreatment was observed [F(2,36) = 0.59, N.S.], but a prepulse intensity \( \times \) pretreatment \( \times \) treatment interaction was found [F(4,72) = 2.74, P < .036]. The probable source of this interaction was the statistically insignificant tendency of pirenzepine to augment PPI in saline-treated animals but further decrease PPI in PCP-treated rats (fig. 5). Thus the M₁ muscarinic antagonist also did not block the deficit in PPI produced by PCP.

No effects were observed on startle magnitude (table 1).

Effect of GABA-A antagonist on PCP-induced behavior. Figure 6 shows the results of the final experiment. In experiment 6, PCP was again found to disrupt PI, as indicated by a main effect of treatment [F(1,42) = 39.9, P < .001] and significant post-hoc comparisons between PCP- and saline-treated groups (P < .01, all three prepulse intensities). In contrast, neither a main effect of pretreatment [F(2,42) = 1.60, N.S.] nor any interaction between factors was indicated by ANOVA. Thus PCP disrupted PI, but this effect was not prevented by the GABA-A antagonist pirenzepine.

Analysis of variance of the startle magnitude data revealed a main effect of PCP treatment [F(1,42) = 9.72, P < .004]. Although post-hoc analyses failed to reveal significant differences between any of the treatment groups, it is evident from examination of the mean values that PCP tended to increase startle magnitude, irrespective of pirenzepin pretreatment (table 1).

Discussion

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Second, the selective alpha-1 noradrenergic antagonist prazosin prevented the PCP-induced deficit in PPI. This effect was produced by multiple prazosin doses, was observed over multiple prepulse intensities and was replicated in a second separate experiment. Finally, selective antagonists for either alpha-2 (RX821002), muscarinic M1 (pirenzepine) or GABA-A (pitrazepin) receptors had no effect on the disruption of PPI produced by PCP. Taken together, these results indicate strongly that the impairment of sensorimotor gating induced by PCP involves the presumably indirect activation of alpha-1 adrenergic receptors, but probably not alpha-2, M1 or GABA-A receptors.

To the best of our knowledge, this is the first report of the blockade of PCP-induced deficits in PPI by an antagonist of noradrenergic receptors. The present findings are in agreement with a recent study that found that locomotor hyperactivity produced by dizocilpine (another noncompetitive NMDA antagonist that also disrupts PPI) is prevented by prazosin in a dose range identical to that of the present studies (Mathe et al., 1996). Furthermore, depletion of NE by the selective neurotoxin N-(2-chloroethyl-N-ethyl-2-bromo-benzylamine) (DSP4) has been reported to prevent both PCP- and amphetamine-induced disruptions in sensory gating as assessed by auditory evoked potentials, which suggests that noradrenergic systems could indeed play a role in central gating mechanisms (Adler et al., 1988; Miller et al., 1992). Deficits in auditory gating produced by PCP have also been reported to be blocked by the nonselective alpha adrenergic antagonist phentolamine (Stevens et al., 1991). The results of the present studies provide further strong support for the hypothesis that the behavioral effects of noncompetitive NMDA antagonists are mediated partially, though not necessarily directly, by alpha-1 adrenergic receptors.

The prazosin-induced antagonism of PCP effects was not accompanied by significant increases in base-line levels of PPI, nor was it altered by calculating PPI as a difference score (PULSE-ALONE value − PREPULSE + PULSE value) rather than as a percentage (data not shown). In addition, the disruption of PPI that was produced by the DA agonist

| Exp. 3 | 2.7 ± 0.9 | 15.4 ± 3.9<sup>a</sup> | 1.9 ± 0.7 | 7.8 ± 2.2 | 2.1 ± 6.7 | 7.5 ± 1.1 |

<sup>a</sup> VEH = vehicle, APO = apomorphine, PRAZ = prazosin. Values represent mean ± S.E.M.

<sup>b</sup> P < .01, compared to VEH/VEH group.
apomorphine was not antagonized by prazosin, even at the highest dose. These findings, taken together, indicate that the antagonism of PCP-induced deficits in PPI by prazosin cannot be attributed to a generalized improvement in either basal or deficient PPI, nor can it be explained simply by changes in startle reactivity. Thus the prazosin antagonism of the PPI deficit appears to be a selective effect on sensorimotor gating abnormalities produced by PCP. It could be suggested that the failure of prazosin to prevent apomorphine-induced deficits in PPI was due to the utilization of doses that were ineffective against this dose of apomorphine. However, the efficacy of prazosin in antagonizing both the increase in startle reactivity (table 1) and that in general activity (e.g., NO STIMULUS scores, table 2) produced by apomorphine in the same animals argues against this possibility. Furthermore, the antagonism by prazosin of the apomorphine-induced increase in startle magnitude is consistent with a previous report that utilized the same dose range as in the present studies (Davis et al., 1985). The effects on NO STIMULUS scores extend the earlier finding that apomorphine increases this measure of motor activity and that this effect can be antagonized by haloperidol (Mansbach et al., 1988).

In contrast to the results with prazosin, selective antagonists for alpha-2, M1 and GABA-A receptors failed to affect PCP-induced deficits in PPI. Although these compounds were without effect in the present studies, it should be noted that the dose ranges utilized are sufficient to achieve bioactivity in other behavioral assays (Siviy et al., 1994; Bymaster et al., 1993). The present set of results further extends the previous finding that selective D1, D2, and 5-HT2 antagonists do not block the disruption in PPI produced by PCP (Bakshi et al., 1994; Keith et al., 1991) and suggests that the effects of clozapine-like antipsychotics in reducing PCP-induced deficits in PPI are probably not derived from antagonism of these receptors. This notion is corroborated by the finding that Seroquel, a novel putative atypical antipsychotic with relatively low affinity for muscarinic, dopaminergic and serotonergic receptors compared with adrenergic receptors (Bymaster et al., 1996), produces a robust blockade of the disruption in PPI produced by PCP (Swerdlow et al., 1996). On the other hand, the locomotor-activating effects of PCP and other noncompetitive NMDA antagonists can be prevented by DA antagonists, which suggests that the mechanisms that bring about the antagonism of sensorimotor gating deficits are distinct from those that mediate general behavioral activation (Hoffman, 1992).

Antipsychotics such as clozapine, olanzapine and Seroquel have been reported to antagonize PCP-induced deficits in PPI (Bakshi et al., 1994; Bakshi and Geyer, 1995; Swerdlow et al., 1996). A shared feature of these compounds is a high affinity for alpha-1 adrenergic receptors (Moore et al., 1993; Coward et al., 1989; Saller and Salama, 1993). In fact, a recent direct comparison of the alpha-1 binding profiles of these compounds revealed that clozapine and Seroquel have identical affinity for the alpha-1 receptor ($K_i = 7 \text{ nM}$) and that this value is approximately half that of olanzapine ($K_i = 19 \text{ nM}$) (Bymaster et al., 1996), which indicates that clozapine and Seroquel are potentially 2-fold more potent as alpha-1 antagonists than olanzapine. Interestingly, the relative doses of these antipsychotics that are required to antagonize PCP-induced deficits in PPI roughly correspond to this ratio; clozapine and Seroquel exhibit blockade at a dose (5 mg/kg) that is half the optimal olanzapine dose (Bakshi et al., 1994; Bakshi and Geyer, 1995; Swerdlow et al., 1996). Seroquel has roughly a 3- to 10-fold higher affinity for alpha-1 than for 5-HT2 receptors (Saller and Salama, 1993; Bymaster et al., 1996). This difference is in contrast to clozapine and olanzapine, which, depending on the assay conditions, show either greater or equal affinity for 5-HT2 compared with alpha-1 sites (Moore et al., 1994; Leysen et al., 1993). Previous research has suggested that 5-HT2 antagonists may worsen the PPI deficit produced by PCP (Bakshi et al., 1994). In conjunction with the present finding of prazosin-induced blockade of PCP-induced deficits, this neurochemical profile raises the possibility that the alpha-1 component of certain antipsychotics is responsible for their antagonism of PCP-induced deficits in PPI but that the 5-HT2 component, which is more pronounced with clozapine and olanzapine, mitigates the alpha-1 effect, resulting in a partial rather than full blockade of the PCP effect. Indeed, Seroquel has been shown to produce a nearly complete antagonism of the PCP-induced deficit in PPI, mirroring the effect of prazosin in the present studies, whereas clozapine and olanzapine antagonize the loss of PPI only by roughly 50% (Bakshi et al., 1994; Bakshi and Geyer, 1995; Swerdlow et al., 1996). Future studies correlating central receptor occupancy with efficacy for blocking PCP-induced deficits in PPI are needed to test this hypothesis directly. Nonetheless, the present findings strongly suggest that clozapine-like antipsychotics might reduce PCP-induced deficits in PPI in large part through antagonism of alpha-1 adrenoceptors. In contrast, clozapine, olanzapine and Seroquel seem to block apomorphine-induced deficits in PPI not through alpha-1 antagonism, but rather via the blockade of DA receptors, because apomorphine-induced deficits in PPI are not blocked by prazosin but can be prevented by haloperidol (Mansbach et al., 1988; Swerdlow et al., 1991). One mystery that remains to be solved is why remoxipride, a relatively selective D2 antagonist that does not possess appreciable alpha-1 affinity, has been reported to block PCP-induced deficits in PPI (Johansson et al., 1994a).

The present results provide solid evidence for an involvement of the noradrenergic system in the PPI-disruptive effects of PCP. Future studies using selective NE-depleting agents could examine the obligatory nature of NE in this effect by investigating whether PCP-induced deficits in PPI could be prevented by the loss of NE. In addition, it will be of interest to determine whether direct alpha-1 agonists disrupt PPI. The precise mechanism by which PCP influences noradrenergic transmission and subsequently alters PPI remains to be determined. PCP increases the firing of DA-containing cells in the ventral tegmental area (French, 1994). Both the increase in DA release and the enhancement of locomotor activity produced by dizocilpine are antagonized by prazosin, which suggests that one mode of functional interaction between noncompetitive NMDA antagonists and noradrenergic systems occurs through the presynaptic modulation of dopaminergic transmission (Svensson et al., 1995; Mathe et al., 1996). Although the dose range of prazosin in the present studies is similar to that used by Svensson and colleagues, it is unlikely that the blockade of the PCP-induced deficit in PPI is mediated by this DA-dependent mechanism, because, in contrast to PCP-induced hyperactivity,
PCP-induced disruptions of PPI cannot be reduced by DA antagonism (Bakshi et al., 1994; Swerdlow et al., 1996; Keith et al., 1991; French and Vantini, 1984). Some of the anatomical sites that mediate the disruption of PPI by noncompetitive NMDA antagonists have been determined (Bakshi and Geyer, 1996), but the brain regions subserving the blockade of this disruption by prazosin remain to be identified. Studies are currently being conducted to delineate the specific neuroanatomical circuitry involved in the interaction between noncompetitive NMDA antagonists and alpha-1 receptors in the modulation of PPI.

It is interesting to note that elevations in NE have been found in the cerebrospinal fluid, plasma or brain tissue of schizophrenia patients, an observation that offers some evidence for disturbances of the noradrenergic system within this psychiatric population (Bird et al., 1979; Breier et al., 1990; Farley et al., 1978; Hornykiewicz, 1982; Kemali et al., 1990; Kleinman et al., 1979; van Kammen et al., 1989). One clinical study, which presumably tested the notion that a state of hypernoradrenergia might be involved in schizophrenia, examined the effects of alpha-1 receptor blockade by prazosin in schizophrenia patients, but did not reveal a significant amelioration of psychotic symptoms (Hommer et al., 1984). It should be noted, however, that the sample was relatively small and included only patients who were subsequently found to improve with administration of “traditional” antipsychotics (e.g., DA antagonists, or neuroleptics). Given that some schizophrenia patients do not respond to DA antagonists but do respond to atypical antipsychotics such as clozapine (Kane et al., 1988), it is possible that different neural substrates are involved in neuroleptic-responsive than in neuroleptic-refractory schizophrenia. Indeed, it may be that some property other than DA receptor antagonism underlies the therapeutic efficacy of clozapine in neuroleptic-resistant schizophrenia patients. Actions at alpha adrenergic systems have been suggested previously to be an important component in the effects of certain antipsychotic medications (Baldessarini et al., 1992; Breier, 1994; Cohen and Lipinski, 1986; Prinssen et al., 1994; Svensson et al., 1995). The results of the present studies provide further evidence for a critical role of the noradrenergic system, and in particular the alpha-1 receptor, in some of the behavioral effects of certain antipsychotics. The present studies show that, like clozapine and related antipsychotics, prazosin prevents PCP-induced PPI deficits. It is possible that the alpha noradrenergic mechanism implicated by the present findings is important in the gating abnormalities exhibited by only a particular subset of schizophrenia patients. Therefore, it could be predicted that prazosin might be effective in treating this subgroup of patients, even though it failed to ameliorate the symptoms of neuroleptic-responsive patients (Hommer et al., 1984). Thus future studies might explore its potential utility in the treatment of either certain neuroleptic-resistant schizophrenia patients or in PCP-induced psychotic states.

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