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Do D_1/D_2 Interactions Regulate Prepulse Inhibition in Rats?

Fang-Jung Wan, M.D., Ph.D., Navid Taaid, and Neal R. Swerdlow, M.D., Ph.D.

Prepulse inhibition (PPI) of the startle reflex is an operational measure of sensorimotor gating that is reduced in schizophrenia patients and in dopamine (DA)–activated rats. We previously found that PPI is disrupted by systemic administration of the D_2 agonist quinpirole, but not by the D_1 agonist SKF 38393. In this report we further characterize the D_1 and D_2 substrates and their potential interactions in the regulation of PPI in rats. PPI is reduced by concomitant administration of the D_1 agonist SKF 38393 (5 mg/kg; relative affinity D_1:D_2 = 50:1) and by a subthreshold dose (0.1 mg/kg) of the D_2 agonist quinpirole, but not by either drug given alone at these doses. Pretreatment with the D_2 antagonist raclopride (0.05 mg/kg), but not the D_1 antagonist SCH 23390 (0.05 mg/kg), blocks the SKF 38393/quinpirole synergistic reduction of PPI. The relative D_1 agonist SKF 82958 (5 mg/kg; relative affinity D_1:D_2 = 10:1) disrupts PPI, and this effect of SKF 82958 is reversed by the D_2 antagonist raclopride but not by the D_1 antagonist SCH 23390. Consistent with a recent report (Hoffman and Donovan 1994), the PPI-disruptive effects of the D_1/D_2 agonist apomorphine (0.5 mg/kg) could be blocked by pretreatment with the D_1 antagonist SCH 23390. Surprisingly the PPI-disruptive effects of quinpirole are also opposed by pretreatment with SCH 23390. Our present findings confirm that D_2 receptors are important for the regulation of PPI in rats, but they also suggest that there exists a synergistic interaction between D_1 and D_2 substrates in the regulation of PPI. D_1 receptors might modulate PPI in a "rate-dependent" manner in which tonic D_1 activity is essential for the full manifestation of the D_2-mediated modulation of PPI. However, D_1 receptors do not appear to participate in the modulatory mechanisms of sensorimotor gating as an independent substrate.
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KEY WORDS: *Dopamine; Quinpirole; SKF 38393; SKF 82958; Sensorimotor gating; Startle*

The acoustic startle reflex is a coordinated contraction of the skeletal or facial muscles in response to an abrupt, intense noise (Ison and Hoffman 1983). The startle reflex is controlled by a simple brain circuit consisting of three synapses linking the auditory nerve with spinal motor neurons (Davis et al. 1982; Lingenhohl and Friauf 1994). Despite its simplicity, startle exhibits several forms of plasticity modulated by higher brain structures. One form of plasticity, prepulse inhibition (PPI), is the normal suppression of the startle reflex when the intense startling stimulus is preceded 30 to 500 ms by a weak prestimulus (Hoffman and Ison 1980). PPI can be measured automatically in humans and rats by using identical stimulus parameters, and provides an operational measure of sensorimotor mechanisms that presumably act to filter, or gate, exteroceptive stimuli (Geyer et al., 1990; Braff et al., 1992). More important, schizophrenia-spectrum patients demonstrate less PPI than do control subjects (Braff et al. 1978; Cadenhead et al. 1993). A similar reduction of PPI occurs in rats after manipulations that increase dopaminergic activity, particularly within the mesolimbic dopamine (DA) system, and after systemic administration of DA agonists (Mansbach et al. 1988; Swerdlow et al. 1990; Wan et al., 1994).

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In addition, the ability of neuroleptics to restore PPI in DA agonist-treated rats correlates significantly with their antipsychotic potency (Swerdlow et al. 1991). Thus, the DA agonist-induced disruption of PPI in rats may provide an animal model to study the neural basis of deficient sensorimotor inhibition in neuropsychiatric patients (Braff et al. 1992; Swerdlow et al. 1994a).

Five distinct dopamine receptor subtypes have been identified in the brain. On the basis of pharmacological properties and amino acid homologies, these five subtypes have been divided into two subfamilies: D_1-like and D_2-like receptors (Sibley et al. 1993). D_1 receptors are capable of stimulating adenylate cyclase, whereas D_2 receptors either inhibit adenylate cyclase or are independent of its function (Kebabian and Calne 1979). We have demonstrated that of PPI is disrupted in rats after systemic administration of the D_2 agonist quinpirole and after infusion of quinpirole directly into the nucleus accumbens (NAC) (Wan and Swerdlow 1993).

There are conflicting reports regarding the role of D_1 receptors in the modulation of PPI. Previous studies from our group and others suggest that D_1 receptors do not appear to modulate PPI potently (Peng et al. 1990; Schwarzkopf et al. 1993; Swerdlow et al. 1991, 1994a). A recent study, however, indicates a possible role of the D_1 receptors in the regulation of PPI based on the finding that the PPI-disruptive effects of the D_1/D_2 agonist apomorphine are opposed by the D_1 antagonist SCH 23390 (Hoffman and Donovan 1994). In addition, it is still under discussion whether D_1 and D_2 receptors affect behavior by synergistic or opposing interactions, or by independent mechanisms (Clark and White 1987). Some preliminary findings suggest that D_1 and D_2 receptors interact synergistically to reduce PPI (Peng et al. 1990). In that study, PPI was reduced by concomitant administration of a "subthreshold" dose of the D_2 agonist quinpirole together with the D_1 agonist SKF 38393. In the present study, we attempted to clarify the role of D_1 and D_2 substrates in the regulation of PPI, and to identify any synergistic properties between D_1 and D_2 substrates in the modulation of the sensorimotor gating in rats.

MATERIAL AND METHODS

Animals

Male Sprague-Dawley rats (250–350 g) were housed in groups of two to three and maintained on a reverse 12-hour light/dark schedule (lights off at 0700) with food and water provided ad libitum. Behavioral testing occurred between 0900 and 1500 during the dark phase when acoustic startle is less variable and more robust (Davis and Sollberger 1971). Animals were handled within 3 days of arrival and daily thereafter.

Apparatus

Each of four startle chambers (SR-LAB, San Diego Instrument, San Diego, CA) was housed in a sound-attenuated room with a 60-dB(A) ambient noise level and consisted of a Plexiglas cylinder 8.2 cm in diameter resting on a 12.5 × 25.5-cm Plexiglas frame within a ventilated enclosure. Acoustic noise bursts were presented via a speaker mounted 24 cm above the animal. A piezoelectric accelerometer mounted below the Plexiglas frame detected and transduced motion within the cylinder. The delivery of acoustic stimuli was controlled by the SR-LAB microcomputer and interface assembly that also digitized (0-4095), rectified, and recorded stabilimeter reading, with one hundred 1-ms readings collected beginning at stimulus onset. Startle amplitude was defined as the average of the 100 readings. Background noise and all acoustic stimuli were delivered through one Radio Shack Supertweeter (frequency response predominantly between 5 and 16 KHz) in each chamber. Stimulus intensities and response sensitivities were calibrated to be nearly identical in each of the four startle chambers (maximum variability <1% of stimulus range and <5% of response ranges), and chambers were also balanced across all experiment groups. Sound levels were measured and calibrated with a Quest Sound Level Meter, A scale (relative to 20 $\mu N/M^2$), with the microphone placed inside the Plexiglas cylinder; response sensitivities were calibrated using an SR-LAB Startle Calibration System.

Test Session

Testing began 7 to 10 days after arrival. First of all, each rat was placed in a startle chamber with 70-dB(A) background noise and 5 minutes later was exposed to seventeen 118-dB(A), 40-ms broad-band bursts ("P-ALONE") with a 15-sec intertrial interval. Rats were then divided into groups matched for mean amplitude on these trials. One to 3 days after the matching session, rats were tested in one of two startle sessions. Both startle sessions began with a 5-min acclimation period with a 70-dB(A) background noise.

One session was designed to study the effects of SCH 23390 on the apomorphine or quinpirole disruption of PPI elicited by relatively intense prepulses. Because of its simple design with a single, relatively intense [15-dB(A) above background] prepulse condition, this session is suitable for direct dose-response comparisons and has been used previously to demonstrate the ability of typical and atypical antipsychotics to restore PPI in apomorphine-treated rats (Swerdlow et al. 1994b). In this session, rats were exposed to two types of stimuli: P-ALONE, and a prepulse that was 15 dB(A) above background [a 85-dB(A), 20-ms broad-band burst presented 100 msec prior to P-ALONE]. The session in-

cluded three trial types: P-ALONE, P-ALONE preceded by the 15-dB prepulse, or no stimulus ("NOSTIM"), presented in pseudorandom order, with a variable intertrial interval averaged 15 seconds.

A second session was used to assess the effects of D_1 and D_2 agonists and antagonists on PPI over a range of prepulse intensities. In this session, rats were exposed to four types of stimuli: a startle pulse [P-ALONE: a 118-dB(A), 40-ms broad-band burst] and three types of prepulses [3, 5, or 10 dB: a 73-, 75-, or 80-db(A), 20-ms broad-band burst] presented 100 msec prior to the startle pulse. The session included five trial types: P-ALONE, each of the three prepulse trials followed by P-ALONE, or no stimulus (NOSTIM). For each test session, 50 trials (10 P-ALONE, 10 NOSTIM, and 10 of each prepulse trial types) were presented in pseudorandom order with a variable intertrial interval that averaged 15 seconds. PPI was defined as: [100 − (startle amplitude on prepulse trials/startle amplitude on P-ALONE trials) × 100]. Using this description of PPI, a high degree of sensorimotor gating is reflected in a high percentage PPI value, whereas less gating results in a small percentage PPI value.

Drug Treatment

Drugs were given subcutaneously with injection volume 1 ml/kg except SKF 82958, which was given intraperitoneally in 2 ml/kg. Dopamine agonists and antagonists were administered 5 and 20 minutes prior to testing, respectively. Mixed designs using drug as the between-subject factor and prepulse intensity as the within-subject factor were used to determine the interactive effects of SKF 38393 and quinpirole, as well as those of SKF 82958 and quinpirole. To study the effects of dopamine D_1 and D_2 antagonists on the disruption of PPI by coadministered D_1/D_2 agonists, rats were divided into two groups and each group was tested with one of the antagonists, with combined D_1/D_2 agonists as the within-subject factor and the antagonist group as the between-subject factor. A between-subject design was applied to assess the effects of pretreatment with raclopride or SCH 23390 on the SKF 82958 disruption of PPI. To study the effects of SCH 23390 on the apomorphine or quinpirole disruption of PPI, rats were tested twice with dose SCH 23390 as the between-subject factor and dose apomorphine or quinpirole as the within-subject factor. Test sessions were typically separated by 4 days, with dose order balanced across sessions.

Data Analysis

Startle data were analyzed by mixed design analyses of variance (ANOVAs), with specific comparisons noted in each experiment. Alpha was 0.05.

RESULTS

Experiment 1: Effects of Systemic Coadministration of SKF 38393 and Quinpirole on PPI

The effects of coadministration of SKF 38393 (5 mg/kg SC) and quinpirole (0.1 mg/kg SC) on PPI are seen in Figure 1. In this study a subthreshold dose of quinpirole, based on our previous data, was used to study the possible synergistic interaction of D_1 and D_2 agonists. A two-way ANOVA using drug as a between-subject factor (including four groups: vehicle/saline, $n = 10$; vehicle/quinpirole, $n = 10$; SKF 38393/saline, $n = 11$; SKF 38393/quinpirole, $n = 10$) with repeated measures on prepulse type revealed that systemic administration of either SKF 38393 or quinpirole did not significantly disrupt PPI [$F = 1.11$, 0.22, respectively, NS], which is consistent with our previous report (Wan and Swerdlow 1993). Coadministration of these doses of SKF 38393 and quinpirole, however, significantly reduced PPI [$F(1,37) = 5.43$, $p = .02$]. There was a significant effect on prepulse type [$F(2,74) = 8.75$, $p < .001$], but no significant drug × prepulse type interaction ($F < 1$ for all groups). There was a significant increase of P-ALONE amplitude (data not shown) following coadministration of SKF 38393 and quinpirole [$F(1,18) = 8.53$, $p < .01$]. P-ALONE amplitude did not correlate significantly with PPI at any prepulse condition (Spearman analysis R = −0.43, −0.57, −0.47; NS). There was

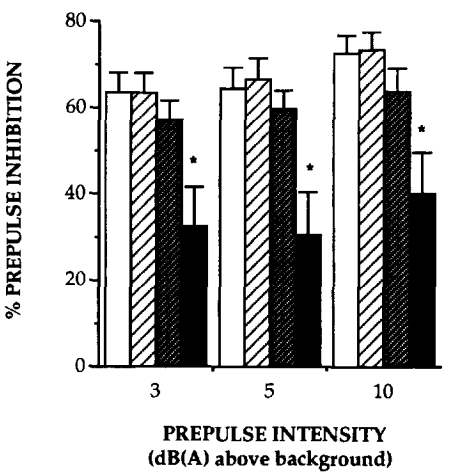

Figure 1. Effects of systemic administration of the D_1 agonist SKF 38393 (5 mg/kg), the D_2 agonist quinpirole (0.1 mg/kg), or coadministration of both drugs on PPI of the acoustic startle in rats. PPI was reduced after concomitant injection of SKF 38393 and a subthreshold dose of quinpirole (*$p < .01$, significantly less than the vehicle treatment group, by Tukey comparison after significant main effect of treatment by ANOVA). Neither quinpirole nor SKF 38393 given alone significantly reduced PPI at these doses. Dose SKF 38393/dose quinpirole: 0/0 mg/kg; *hatched bars*: 0/0.1 mg/kg; *double hatched bars*: 5/0 mg/kg; and *solid bars*: 5/0.1 mg/kg.

no significant effect of either SKF 38393 or quinpirole administered alone on P-ALONE amplitude ($F = 3.06$ and $F < 1$, respectively, NS).

Experiment 2: Effects of Pretreatment with SCH 23390 or Raclopride on the SKF 38393/ Quinpirole-induced Disruption of PPI

This experiment examined the effects of pretreatment with either the D_1 antagonist SCH 23390 (0.05 mg/kg SC) or the D_2 antagonist raclopride (0.05 mg/kg SC) on the reduction in PPI caused by the concomitant administration of SKF 38393 and quinpirole. These doses of the D_1 and D_2 antagonists show significant effects on PPI (Hoffman and Donovan 1994; Swerdlow et al. 1991). The results are seen in Figure 2. A two-way ANOVA using group (SCH 23390, $n = 16$ vs. raclopride, $n = 8$) as a between-subject factor and treatment (saline + SKF 38393/quinpirole or antagonist + SKF 38393/quinpirole) as well as prepulse type as within-subject factors revealed a significant effect on group [$F(1,22) = 5.49$, $p = .03$] and a significant effect of treatment [$F(1,22) = 4.54$, $p = 0.04$]. There was a significant group × prepulse-type interaction [$F(2,44) = 5.46$, $p < .01$], but no significant effect of prepulse type or any two- or three-way interactions (all $p > .05$). The PPI-disruptive effects of coadministration of SKF 38393/ quinpirole were reversed by pretreatment with the D_2 antagonist raclopride, but not by pretreatment with the D_1 antagonist SCH 23390. Post hoc independent ANOVAs revealed that in rats treated with SKF 38393/quinpirole, PPI was significantly restored by raclopride [$F(1,7) = 7.13$, $p = .03$] (Figure 2A), but not by SCH 23390 [$F(1,15) < 1$, NS]; (Figure 2B). These findings were replicated by another study (data not shown) using a higher dose of SCH 23390 (0.1 mg/kg SC). In this study SCH 23390 did not significantly reverse the synergistic disruption of PPI by SKF 38393/quinpirole [$F(1,15) <$ NS].

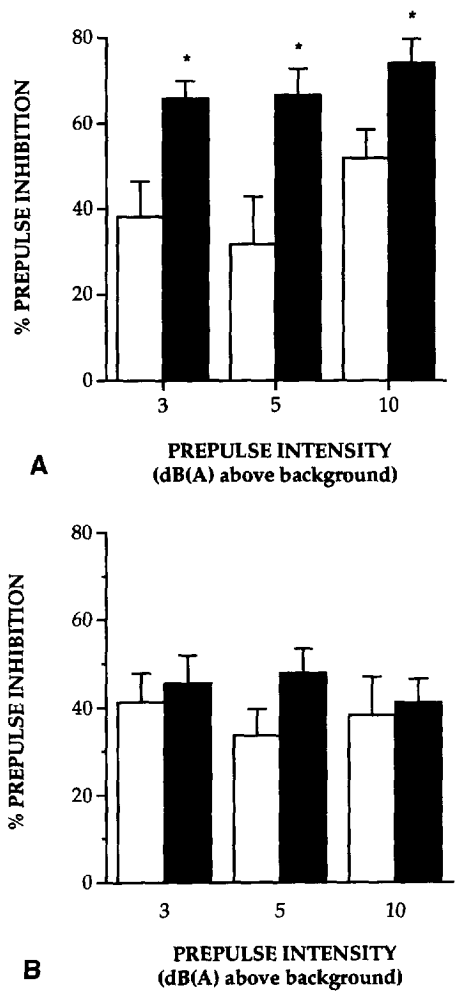

Figure 2. Effects of pretreatment with the D_2 antagonist raclopride (**A**) or the D_1 antagonist SCH 23390 (**B**) on the synergistic reduction of PPI after coadministration of SKF 38393 (5 mg/kg)/quinpirole (0.1 mg/kg). The reduction of PPI after coadministration of SKF 38393/quinpirole was reversed by raclopride (0.05 mg/kg), but not by SCH 23390 (0.05 mg/kg). Similar findings were obtained in a separate group of rats, using a higher dose of SCH 23390 (0.1 mg/kg). (*$p < .01$, significantly less than the saline pretreatment group by individual ANOVA after significant main effect of raclopride by ANOVA). Part *A*, *open bars*: saline/SKF 38393 + quinpirole; *solid bars*: raclopride/SKF 38393 + quinpirole. Part *B*, *open bars*: saline/SKF 38393 + quinpirole; *solid bars*: SCH 23390/ SKF 38393 + quinpirole.

Experiment 3: Effects of Systemic Coadministration of SKF 82958 and Quinpirole on PPI

To further explore possible D_1/D_2 interactions in the regulation of PPI, we studied the effects of the D_1 agonist SKF 82958 (5 mg/kg IP) on PPI, either given alone or in conjunction with a presumed subthreshold dose of quinpirole (0.1 mg/kg SC). In this experiment rats were tested with the identical drug doses and test session used in Experiment 2, except that the D_1 agonist was SKF 82958. A two-way ANOVA using drug as a between-subject factor (including four groups: vehicle/ saline, $n = 7$; vehicle/quinpirole, $n = 8$; SKF 82958/ saline, $n = 8$; SKF 82958/quinpirole, $n = 8$) with repeated measures on prepulse type revealed that, in contrast to the effects of SKF 38393 on PPI in Experiment 2, SKF 82958 significantly reduced PPI [$F(1,27) =$ 10.55, $p = .001$]. Quinpirole given alone at this dose did not significantly reduce PPI [$F(1,27) = 4.03$, NS], but a strong trend was evident (Figure 3). There was a significant effect of prepulse type [$F(2,54) = 10.67$, $p < .001$], but no significant SKF 82958 × quinpirole interaction [$F(1,27) < 1$, NS] or other two- or three-way interactions [$F < 1$ all comparisons]. In comparison to PPI in vehicle-treated rats, PPI was significantly reduced following administration of SKF 82958 alone or following the co-

administration of SKF 82958 and quinpirole [$F(1,13)$ = 10.62 and 11.27; respectively, both at $p < .01$], analyzed by independent ANOVAs (Figure 3). An individual ANOVA comparing PPI in the vehicle/quinpirole group versus the SKF 38393/quinpirole group revealed that SKF 82958 reduced PPI in quinpirole-treated rats [$F(1,14) = 4.62$, $p < .05$]. P-ALONE amplitude was not significantly changed by SKF 82958 or quinpirole given separately [$F(1,27)$ = 1.03 and 1.38, respectively, NS] or together [$F(1,27) = 2.74$, NS].

Experiment 4: Effects of Pretreatment with SCH 23390 or Raclopride on the SKF 82958-induced Disruption of PPI

The differential effects between SKF 82958 and SKF 38393 on PPI may result either from the stronger capability of SKF 82958 to stimulate a second messenger cascade, or perhaps more likely from the nonselective activation of both D_1 and D_2 receptors by SKF 82958 (also see Discussion). In order to better understand the properties of the PPI-disruptive effect of the relative D_1 agonist SKF 82958, this effect of SKF 82958 was studied after pretreatment with either the D_1 antagonist SCH 23390 (0.05 mg/kg SC) or the D_2 antagonist raclopride (0.05 mg/kg SC). In accordance with the results of Experiment 3, a two-way ANOVA using drug (four groups, $n = 6$ for each group) as a between-subject factor, with repeated measures on prepulse type, revealed that SKF 82958 at the dose of 5 mg/kg significantly reduced PPI [$F(1,10) = 6.02$, $p = .03$] without significantly changing P-ALONE amplitude [$F(1,10) < 1$, NS]. Separate analyses in raclopride- and SCH 23390-pretreated rats revealed no significant main effect of raclopride [$F(1,10) = .20$, NS], but a significant interaction of raclopride × prepulse type [$F(2,20) = 4.42$, $p = .02$]. Post hoc comparison revealed that raclopride significantly reversed the PPI-disruptive effects of SKF 82958 for the 3-dB prepulse trials [$F(1,10) = 11.10$, $p < .01$; Figure 4]. In contrast, there was no significant main effect of SCH 23390 [$F(1,10) < 1$, NS], and no significant interaction of SCH 23390 × prepulse type [$F(2,20) < 1$, NS].

Experiment 5: Effects of Pretreatment of SCH 23390 on the Apomorphine-induced Disruption of PPI

Although the data from preceding studies do not suggest that there is a direct D_1 modulation of PPI, a recent report suggests that D_1 receptors play a role in the modulation of PPI in rats (Hoffman and Donovan 1994). In this study, the D_1 antagonist SCH 23390 attenuated the apomorphine-induced disruption of PPI. This finding, however, contrasts with a previous report by our group, which failed to demonstrate a similar effect of SCH 23390 at comparable doses (Swerdlow et al. 1991). Hoffman and Donovan (1994) suggested that the different effects of SCH 23390 across laboratories might be due to methodological differences, including the use of different test sessions and prepulse types. In the original report (Swerdlow et al. 1991), the difference score (ampli-

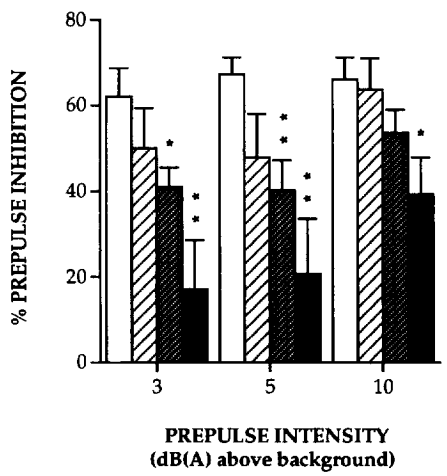

Figure 3. Effects of systemic administration of the D_1 agonist SKF 82958 (5 mg/kg), the D_2 agonist quinpirole (0.1 mg/kg), or coadministration of both drugs on PPI. The PPI was disrupted after systemic injection of SKF 82958, but not after quinpirole given alone. SKF 82958 also reduced PPI in quinpirole-treated rats (*$p < .05$, **$p < .01$, significantly less than the vehicle treatment group by Tukey comparison after significant main effect of treatment by ANOVA). Dose SKF 82958/dose quinpirole: *open bars*: 0/0 mg/kg; *hatched, bars*: 0/0.1 mg/kg; *double hatched bars*: 5/0 mg/kg; *solid bars*: 5/0.1 mg/kg.

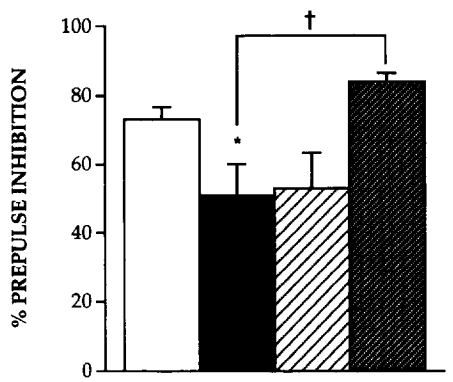

Figure 4. Effects of pretreatment with the D_2 antagonist raclopride or the D_1 antagonist SCH 23390 on the SKF 82958 reduction of PPI. PPI was significantly reduced after systemic administration of SKF 82958 (5 mg/kg; *$p < .05$). This PPI-disruptive effect of SKF 82958 was reversed by pretreatment with raclopride (0.05 mg/kg; †$p < .01$), but not by pretreatment with SCH 23390 (0.05 mg/kg; Tukey comparison after significant interaction of raclopride × prepulse type by ANOVA). Only data from 3-dB prepulse intensity are shown. *Open bars*: saline/vehicle; *solid bars*: saline/SKF 82958; *hatched bars*: SCH 23390/SKF82958; *double hatched bars*: raclopride/SKF 82958.

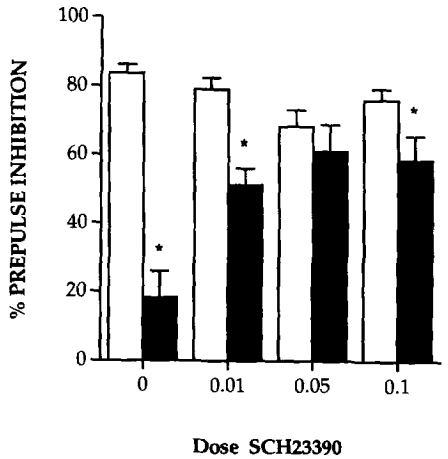

Figure 5. Effects of pretreatment with SCH 23390 (0, 0.01, 0.05, and 0.1 mg/kg) on the apomorphine reduction of PPI. PPI was significantly reduced after systemic administration of apomorphine (0.5 mg/kg; $*p < .01$), by independent ANOVAs after significant main effect of apomorphine and SCH 23390 × apomorphine interaction. In contrast to vehicle pretreated rats, there was no significant effect of apomorphine on PPI when rats were pretreated with 0.05 mg/kg SCH 23390. *Empty bars*: no apomorphine; *solid bars*: 0.5 mg/kg apomorphine.

tude on pulse alone minus amplitude on (prepulse + pulse) trials) was used to quantify PPI instead of a percentage score; conceivably, a difference score analysis might mask the effects of SCH 23390 on PPI when startle amplitude is depressed. This might be particularly relevant since the original report (Swerdlow et al. 1991) used a long, complex test session in which the amplitude-lowering effects of startle reflex habituation were most pronounced.

Thus, we decided to reevaluate this SCH 23390/apomorphine relationship using a brief, simplified test session that detects the ability of several typical and atypical antipsychotics to restore PPI in apomorphine-treated rats (Swerdlow et al. 1994b). The results are seen in Figure 5. A two-way ANOVA using SCH 23390 (dose: 0, 0.01, 0.05, 0.1 mg/kg SC; n = 8, 8, 8, and 10) as a between-subject factor, and apomorphine (0 or 0.5 mg/kg SC) as a within-subject factor revealed an overall significant effect of apomorphine [$F(1,30) = 91.59$, $p < .001$]. Although there was no effect of SCH 23390 on PPI [$F(3,30) = 2.41$ NS], a significant SCH 23390 × apomorphine interaction [$F(3,30) = 16.26$, $p < .001$] was noted. Post hoc individual ANOVAs revealed that PPI was reduced in rats treated with either apomorphine alone or in rats pretreated with SCH 23390 at doses of 0.01 and 0.1 mg/kg ($p < .01$ in all three groups). In contrast, apomorphine did not significantly reduce PPI when rats were pretreated with 0.05 mg/kg SCH 23390 (SCH 23390/vehicle vs. SCH 23390/apomorphine) [$F(1,7) = 1.00$, NS]. Thus, consistent with the report of Hoffman and Donovan (1994), SCH 23390 (0.05 mg/kg) reversed the PPI-reducing effects of apomorphine.

P-ALONE amplitude was decreased following systemic injection of SCH 23390 [$F(3,30) = 3.43$, $p = 0.03$]. There was a nonsignificant trend for apomorphine to increase P-ALONE [$F(1,30) = 2.41$, NS], and the opposing effects of apomorphine and SCH 23390 on startle amplitude resulted in a significant SCH 23390 × apomorphine interaction [$F(3,30) = 3.96$, $p = .017$] (data not shown).

Experiment 6: Effects of Pretreatment with SCH 23390 on the Quinpirole-induced Disruption of PPI

One interpretation of the present results, including the synergistic reduction of PPI by D_1 and D_2 agonists, and the reversal of the PPI-disruptive effects of apomorphine by a D_1 antagonist, is that D_1 receptors serve a permissive role in the PPI-reducing effects of dopamine activation. According to this hypothesis, even though PPI is not altered after direct stimulation of D_1 receptors, the existence of tonic D_1 activity is required for the full expression of the D_2-mediated modulation of PPI. To test this hypothesis, we examined the effects of SCH 23390 on the reduction of PPI by the D_2-family agonist quinpirole. If activity at D_1 receptors permits the manifestation of the D_2 regulation of PPI, we would predict that blockade of D_1 receptors might attenuate the expected PPI-disruptive effects of quinpirole.

We studied two doses of quinpirole: one (0.63 mg/kg) of equal molarity to the dose of apomorphine used in previous experiment, and another (1 mg/kg) that yielded a reduction of PPI that was more comparable to that produced by apomorphine. The results using the lower dose of quinpirole are seen in Figure 6A. A two-way ANOVA using SCH 23390 (dose: 0, 0.01, 0.05, and 0.1 mg/kg SC; n = 8 in each group) as a between-subject factor, and quinpirole (0 or 0.63 mg/kg SC) as a within-subject factor revealed an overall significant effect of quinpirole on the reduction of PPI [$F(1,28) = 21.15$, $p < .001$]. There were no effects of SCH 23390 [$F(3,28) < 1$, NS] or interaction of SCH 23390 × quinpirole [$F(3,28) < 1$, NS]. P-ALONE amplitude was decreased (data not shown) following systemic injection of either SCH 23390 [$F(3,28) = 4.17$, $p = .014$] or quinpirole [$F(1,28) = 12.60$, $p < .01$]. There was no significant interaction of SCH 23390 × quinpirole on P-ALONE [$F(3,28) < 1$, NS].

Using this lower dose of quinpirole, PPI was reduced from 80% (0 mg/kg) to 53% (0.63 mg/kg); this effect was markedly weaker than that produced by a 0.5-mg/kg dose of apomorphine, which lowered PPI from 83% (0 mg/kg) to 18% (0.5 mg/kg). This relatively weak effect of quinpirole might have obscured the detection of any opposing effects by SCH 23390, which restored PPI to 59% both in studies with apomorphine (Experiment 5) and with quinpirole (this experiment).

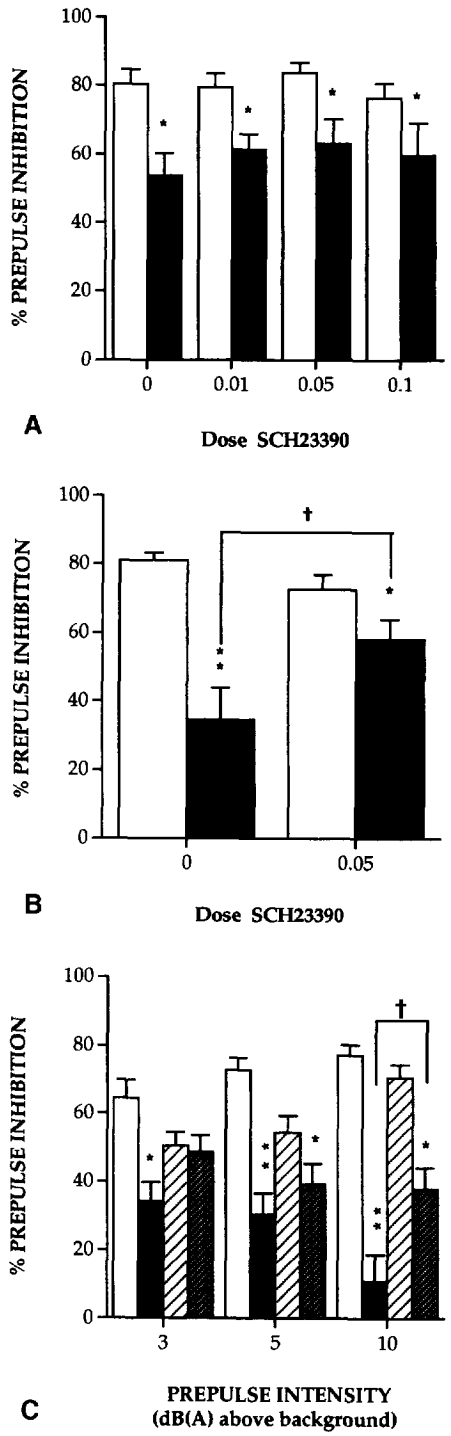

Figure 6. Effects of pretreatment with SCH 23390 on the quinpirole reduction of PPI. (**A**) Effects of pretreatment with SCH 23390 (0, 0.01, 0.05, 0.1 mg/kg) on the PPI reduction by 0.63 mg/kg quinpirole. PPI was significantly reduced by quinpirole (*$p < .01$), by individual ANOVAs after significant main effect of quinpirole; SCH 23390 did not significantly reverse this effect. *Open bars*: no quinpirole; *solid bars*: 0.63 mg/kg quinpirole. (**B**) Effects of pretreatment with SCH 23390 (0.05 mg/kg) on the PPI reduction by 1 mg/kg quinpirole. As in (*A*), PPI was significantly reduced by quinpirole (** $p < .001$, * $p < .01$). In this case, this quinpirole effect was significantly opposed by 0.05 mg/kg SCH 23390 ($^{\dagger}p = .04$, by independent ANOVA after significant SCH 23390 × quinpirole interaction). *Open bars*: no quinpirole; *solid bars*: 1 mg/kg quinpirole. (**C**) Effects of pretreatment with SCH 23390 (0.05 mg/kg) on the PPI reduction by quinpirole (1 mg/kg) in a test session using weaker prepulses (3, 5, and 10 dB). PPI was significantly reduced by quinpirole with all prepulse intensities in rats pretreated with vehicle (**$p < .001$, *$p < .01$), but not with 3-dB prepulses in rats pretreated with SCH 23390. Furthermore, the PPI-disruptive effect of quinpirole with 10-dB prepulses was significantly opposed by SCH 23390 ($^{\dagger}p = .03$). Dose SCH 23390/dose quinpirole: *open bars*: 0/0 mg/kg; *solid bars*: 0/1 mg/kg; *hatched bars*: 0.05/0 mg/kg; *double hatched bars*: 0.05/1 mg/kg.

The effects of 0.05 mg/kg of SCH 23390 on the PPI reduction by a higher dose of quinpirole (1 mg/kg) are seen in Figure 6B. A two-way ANOVA using SCH 23390 (dose: 0, 0.05 mg/kg SC; both $n = 14$) as a between-subject factor, and quinpirole (0 or 1 mg/kg SC) as a within subject factor revealed an overall significant effect of quinpirole [$F(1.26) = 41.43$, $p < .001$]. Although there was no effect of SCH 23390 on PPI [$F(1,26) = 1.16$, NS], a significant SCH 23390 × quinpirole interaction [$F(1,26) = 11.30$, $p < .01$] was noted. Post hoc individual ANOVAs revealed that the effects of quinpirole on PPI were significantly reduced by pretreatment with 0.05 mg/kg SCH 23390 [$F(1,26) = 4.57$, $p = .04$; vehicle/quinpirole vs. SCH 23390/quinpirole groups]. P-ALONE amplitude was decreased (data not shown) following systemic injection of either SCH 23390 [$F(1,26) = 4.28$, $p = .04$] or quinpirole [$F(1,26) = 7.30$, $p = .01$]. There was no significant interaction of SCH 23390 × quinpirole on P-ALONE [$F = (1,26) = 1.49$, NS]. As in both Experiment 5 and the present experiment with the lower dose of quinpirole, SCH 23390 (0.05 mg/kg) increased PPI to approximately 59%. Compared to the relatively weak effects of SCH 23390 on the PPI reduction by the lower dose of quinpirole, this reversal by SCH 23390 was more easily detected when PPI was reduced to approximately 34% by the higher dose of quinpirole. This observation is consistent with the hypothesis that the effects of D_1 blockade on the D_2 modulation of PPI are permissive and "rate dependent." This type of interactive relationship between D_1 and D_2 receptors has been reported in many other paradigms (Longoni et al. 1987; Walters et al. 1987; White and Hu 1993).

We further explored this apparent D_1/D_2 interaction in separate rats using a test session with weaker prepulses [3, 5, and 10 dB(A) above background] that yielded lower "baseline" levels of PPI [approximately 60–75%, compared to 80–85% PPI with 15-dB(A) prepulses, Figure 6C]. A two-way ANOVA using SCH 23390 (dose: 0 and 0.05 mg/kg SC; both $n = 14$) as a between-subject factor and quinpirole (0 or 1.0 mg/kg SC) and prepulse type as within-subject factors revealed an overall significant effect of quinpirole [$F(1,26) = 42.82$, $p <$

.001], a significant SCH 23390 × quinpirole interaction [$F(1,26) = 9.66$, $p < .01$], a significant quinpirole × prepulse type interaction [$F(2,52) = 24.36$, $p < .001$] a significant SCH 23390 × prepulse type interaction [$F(2,52) = 5.96$, $p < .01$], but no significant main effect of SCH 23390 or prepulse type or any other two- or three-way interactions (all $F < 1$, NS). Post hoc independent comparisons between vehicle/saline versus vehicle/quinpirole groups at each prepulse type revealed that quinpirole significantly reduced PPI for all prepulse intensities (all $p < .01$). Post hoc independent comparisons between SCH 23390/saline versus SCH 23390/quinpirole groups at each prepulse type revealed that quinpirole significantly reduced PPI for the 5- and 10-dB trials ($p < .05$ and $p < .001$, respectively) but not for the 3-dB prepulse trials ($F < 1$, NS). Furthermore, SCH 23390 significantly blunted the effects of quinpirole on PPI for the 10-dB prepulse trials ([$F(1,26) = 5.25$, $p = .03$], vehicle/quinpirole vs. SCH 23390/quinpirole).

Post hoc examination also revealed that the significant interaction of SCH 23390 × prepulse type reflected a significant SCH 23390-induced reduction in PPI for the 3- and 5-dB prepulse intensities ($p < .05$ and $p < .01$, respectively). This SCH 23390-induced reduction in PPI is consistent with our previous experience (Swerdlow et al. 1991) with 0.05 mg/kg SCH 23390. This effect appears to be intensity dependent and weakens with higher-intensity prepulses. Still, a trend for similar effects of 0.05 mg/kg SCH 23390 can be seen with 15-dB prepulses in Figures 5 and 6B.

P-ALONE amplitude was decreased (data not shown) following systemic injection of quinpirole [$F(1,26) = 13.29$, $p < .01$]. There was no significant effect of SCH 23390 or interaction of SCH 23390 × quinpirole on P-ALONE [$F(1,26) = 2.26$ and 0.14; NS].

DISCUSSION

We previously reported that PPI is significantly reduced in rats treated with the D_2 agonist quinpirole, but not with the D_1 agonist SKF 38393 (Wan and Swerdlow 1993). Others have reported a possible synergistic interaction between D_1 and D_2 receptors in the regulation of PPI (Peng et al. 1990; Schwarzkopf et al. 1993). A synergism between D_1 and D_2 substrates might be a fundamental property of normal dopaminergic function, in which concomitant stimulation of both D1 and D_2 receptors is required for the full manifestation of certain dopamine-mediated responses (Longoni et al. 1987; Walters et al. 1987; Waddington and Daly 1993). For example, D_2-mediated rotation, stereotypy, and striatal neuron inhibition are enhanced by D_1 agonists, and selective D_1 antagonism prevents or reduces certain behavioral and electrophysiological effects of D_2 agonists (Rouillard and Bedard 1988; Bordi and Meller 1989; White and Hu 1993). In addition, D_1 and D_2 synergistic interaction has been demonstrated at the cellular level (Bertorello et al. 1990; Miomelli et al. 1991).

In the present report, systemic coadministration of the D_1 agonist SKF 38393 (5 mg/kg) and of a subthreshold dose (0.1 mg/kg) of the D_2 agonist quinpirole appears to synergistically disrupt PPI. While pharmacological "synergism" cannot be convincingly demonstrated without more substantial dose-response studies, it would be difficult to account for our findings on the basis of simple additive effects (Figure 1). Our findings are consistent with previous work, in which a higher dose of SKF 38393 (10 mg/kg) was used to explore a possible interaction between D_1 and D_2 receptors regulating PPI in rats (Peng et al. 1990). It also has been reported that D_1 and D_2 antagonists exhibit a synergistic interaction in the regulation of PPI (Schwarzkopf et al. 1993). In the present study, however, PPI lowered to approximately 40% inhibition by the combination of (SKF 38393 + quinpirole) was reversed by pretreatment with the D_2 antagonist raclopride, but not by the D_1 antagonist SCH 23390. Thus, even though quinpirole and SKF 38393 appear to synergistically reduce PPI, we found that this effect was antagonized only by blockade of D_2 receptors.

SKF 82958 was used to further explore possible D_1/D_2 receptor interactions in the regulation of PPI. SKF 82958 is characterized as a full D_1 agonist, with higher intrinsic activity than SKF 38393 in stimulating adenylate cyclase (O'Boyle et al. 1989). SKF 82958 also is more potent than SKF 38393 in eliciting certain behaviors associated with D_1 function (Murray and Waddington 1990; Terry and Katz 1992). On the other hand, SKF 38393 is more D_1 selective in vitro than SKF 82958: there is a 50-fold separation of affinity between D_1 and D_2 receptors for SKF 38393, whereas SKF 82958 has about a 10-fold separation (Andersen et al. 1990; Murray and Waddington 1990). In the present report, SKF 82958 was found to reduce PPI, and coadministration of SKF 82958 and a subthreshold dose of quinpirole further reduced PPI. The PPI-disruptive effects of SKF 82958 were reversed by pretreatment with the D_2 antagonist raclopride, but not by pretreatment with the D_1 antagonist SCH 23390. As with our earlier studies with SKF 38393, these experiments with SKF 82958 suggest that PPI is reduced by combined D_1/D_2 agonism, but that these effects on PPI were antagonized only by blockade of D_2 receptors.

Interestingly, each case in which SCH 23390 failed to oppose the PPI-reducing effects of combined D_1/D_2 agonism occurred when the agonists-reduced levels of PPI did not drop below approximately 40%. In contrast, in Experiment 5, SCH 23390 significantly opposed the effects of apomorphine, which by itself lowered PPI to below 20%. Similarly, SCH 23390 significantly opposed the quinpirole-induced disruption of PPI only when PPI was lowered to approximately 34%. SCH 23390 did

not significantly reverse drug effects on PPI when PPI was reduced to approximately 50% by a lower dose of quinpirole (0.63 mg/kg), nor did it significantly reverse the effects of either (SKF 38393 + quinpirole) or SKF 82958, which lowered PPI to 40% and 50%, respectively. One way to interpret these findings is that D_1 receptors modulate PPI in a "rate-dependent" manner. In such a modulatory role, direct D_1 receptor activation by itself does not potently depress PPI, but complete reduction of PPI by D_2 receptor activation depends on the "permissive" effects of tonic D_1 activity.

A series of studies have shown that PPI is regulated by neural circuitry linking limbic and mesolimbic structures, including the hippocampus and the ventral striatum, with the primary startle circuit, via subpallidal efferents to the pontine reticular formation (Caine et al. 1992; Koch et al. 1993; Swerdlow and Geyer 1993; Wan et al. 1994; Kodsi and Swerdlow 1995). Our present study supports the notion that the dopaminergic regulation of PPI is predominantly mediated by D_2 receptors. We did not find direct evidence supporting an independent D_1 regulation of PPI in intact rats. However, our present data suggest a synergistic interaction between D_1 and D_2 substrates in the regulation of PPI, and a permissive role for D_1 receptors in the D_2-mediated reduction in PPI.

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