Pharmacology Biochemistry and Behavior, Vol. 44, pp. 741–744, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

RAPID COMMUNICATION

Clozapine and Haloperidol in an Animal Model of Sensorimotor Gating Deficits in Schizophrenia

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Received 21 September 1992

SWERDLOW, N. R. AND M. A. GEYER. *Clozapine and haloperidol in an animal model of sensorimotor gating deficits in schizophrenia.* PHARMACOL BIOCHEM BEHAV **44**(3) 741–744, 1993.—Prepulse inhibition (PPI) of the acoustic startle response is a measure of sensorimotor gating that is impaired in both schizophrenic patients and in rats treated with dopamine agonists. The disruption of PPI by the dopamine agonist apomorphine (APO) is reversed by antipsychotic agents, including the atypical antipsychotic clozapine. Across a range of compounds, the ability of antipsychotics to restore PPI in APO-treated rats correlates significantly with their clinical potency. Since few animal models predict antipsychotic potency for clozapine, we further characterized the effects of clozapine and the typical antipsychotic haloperidol on APO-disrupted and baseline PPI in rats. The APO-induced disruption of PPI caused by intense (15 dB over background) prepulses was reversed in a dose-dependent manner by both clozapine and haloperidol. When weak (1–5 dB over background) prepulses were used, clozapine and haloperidol increased baseline PPI in control animals. Both APO-disrupted and baseline PPI may be useful in screening both typical and atypical antipsychotic agents.

Clozapine Haloperidol Apomorphine Startle Schizophrenia

THE AMPLITUDE of the startle reflex is inhibited when the startling stimulus is preceded 30–500 ms earlier by a weak prepulse (5–8). Since "prepulse inhibition" (PPI) provides an operational measure of sensorimotor gating, the deficient PPI observed in schizophrenic patients (1,2) may reflect impaired sensorimotor gating that results in "sensory flooding" and consequent cognitive fragmentation (11). In rats, the disruption of PPI by systemic treatments with dopamine (DA) agonists appears to be mediated by their effects on D_2 receptors (16), most likely within regions of the nucleus accumbens and anterior striatum (14,15). The ability of antipsychotic agents to reverse the PPI-disruptive effects of DA agonists such as apomorphine (APO) correlates significantly ($r = 0.991$) with their clinical potency (17,19). The potential utility of this model as a screening agent for antipsychotic compounds is further supported by the fact that it is specific to clinically effective antipsychotics (13,17,20) and also predicts potency for the atypical antipsychotic clozapine (19). Thus, we have previously reported that clozapine reverses the APO-induced disruption of PPI in rats (19). Furthermore, there is evidence that clozapine normalizes PPI in schizophrenic patients (23). Drugs that fail to restore PPI in APO-treated rats include propranolol (17,20), naloxone (21), buspirone, diazepam and imipramine (13), all of which lack clinical antipsychotic potency.

In our initial report of the PPI-restorative properties of clozapine, we noted an "inverted-U" shaped dose-response function, in which the APO-disruption of PPI was reversed by lower (1–4 mg/kg), but not higher doses of clozapine (19). Findings in this study were difficult to interpret for two reasons. First, because the startle session was complex, with five different stimulus conditions distributed over three time blocks, the results may have reflected interactions between the effects of two drugs, multiple prepulse conditions, startle amplitude (which was significantly decreased by clozapine) and reflex habituation (which was significantly enhanced by clozapine) (19). Second, PPI was analyzed using the difference between the reflex amplitude on startle trials (pulse alone) and the amplitude on trials in which a prepulse preceded the startling pulse (prepulse + pulse). This "difference score"

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measure is confounded in conditions where drugs—like clozapine—significantly depress startle amplitude, because a low-startle amplitude inevitably yields a low-difference score suggestive of decreased PPI. For this reason, the high doses of clozapine (>10 mg/kg) that depressed startle amplitude may have resulted in artifactual decreases in the difference score and thus PPI. Third, the prepulse intensities used in that study [15 dB(A) over background] resulted in maximal "ceiling" levels of PPI, which preclude detection of possible facilitatory effects of drugs on PPI (19).

To overcome these difficulties, we studied the effects of clozapine and the typical antipsychotic haloperidol on APO-disrupted PPI using a simplified paradigm with one prepulse condition and a short session designed to minimize the potentially confounding effects of reflex habituation. A second experiment assessed the effects of clozapine and haloperidol on PPI using weak prepulses [1-5 dB(A) over background] to allow detection of possible facilitatory drug effects on PPI. Data were analyzed using percentage scores, which are less sensitive to the potentially confounding effects of changes in startle amplitude (4).

METHOD

One hundred and thirty male Sprague-Dawley rats (225-250 g) were housed in groups of two to three and maintained on a reversed 12L : 12D schedule (lights off at 0700) with food and water provided ad lib. Behavioral testing occurred during the dark phase, when startle is most robust and least variable (3). Rats were handled individually within 3 days of arrival, and daily thereafter. Some rats were tested in an open field photocell apparatus at least 2 weeks prior to startle testing.

All four startle chambers (SR-LAB, San Diego Instruments, San Diego, CA) were housed in a sound-attenuated room with a 60 dB(A) ambient noise level. Each chamber consisted of a Plexiglas cylinder 8.2 cm in diameter resting on a 12.5 × 25.5 cm Plexiglas frame within a ventilated enclosure. Noise bursts were presented via a speaker mounted 24 cm above the rat. A piezoelectric accelerometer mounted below the Plexiglas frame detected and transduced motion within the cylinder. Stimulus delivery was controlled by the SR-LAB microcomputer and interface assembly, which also digitized (0-4095), rectified, and recorded stabilimeter readings, with 100 one-millisecond 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 reponse predominantly between 5-16 KHz) in each chamber. Stimulus intensities and response sensitivities were calibrated to be nearly identical in each of the startle chambers (maximum variability <1% of stimulus range and <5% of response ranges). Chambers were balanced across all experimental 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.

To study the effects of clozapine and haloperidol on APO-disrupted PPI, testing occurred during two sessions, with 7 d between sessions. Clozapine was dissolved in half volume 0.1 N HCl and diluted to full volume with saline (final pH 5.0-6.0). Haloperidol was dissolved in saline. APO was dissolved in saline with 0.1% ascorbic acid. Each rat was pretreated with one dose of either clozapine (0, 1.5, 3, 6, 9, or 12 mg/kg IP, n = 68) or haloperidol (0, 0.01, 0.05, 0.1, or 0.5 mg/kg SC, n = 40). Injection volume was 1 mg/ml. Ten minutes later, rats were treated with APO (0 or 0.5 mg/kg SC) and then immediately placed into the startle chambers for a 5-min acclimation period with a 65 dB [A] background noise. After the acclimation period, rats were exposed to two types of stimuli: a startle pulse ("PULSE": 118 dB (A) 40-ms broad band burst) and a prepulse that was 15 dB(A) above background [80 dB(A) 20 ms broad band burst] presented 100 ms prior to PULSE. The session included three trial types: PULSE, prepulse followed by PULSE, or no stimulus (NOSTIM). For each session, 63 trials (21 PULSE, 21 prepulse + PULSE and 21 NOSTIM) were presented in pseudorandom order. A variable intertrial interval averaged 15 s. One week later, the procedure was repeated, but APO doses were reversed, with the number of rats receiving each dose of APO balanced over both sessions.

To study the effects of clozapine and haloperidol on "baseline" PPI, rats were tested in three sessions, each separated by 4 days. Rats (n = 12) were pretreated with either vehicle, clozapine (12 mg/kg IP) or haloperidol (0.1 mg/kg SC), prepared as above. Ten minutes later, rats were placed into the startle chambers for a 5-min acclimation period with a 65 dB (A) background noise. After the acclimation period, rats were exposed to six types of stimuli: PULSE or prepulses (20-ms broad-band bursts) that were 1, 2, 3, 4, or 5 dB(A) above background, presented 100 ms prior to PULSE. The session included seven trial types: PULSE, PULSE preceded by one of the five prepulse types, or NOSTIM. For each session, 49 trials (7 PULSE, 7 of each prepulse + PULSE and 7 NOSTIM) were presented in pseudorandom order. A variable intertrial interval averaged 15 s. Four and 8 days later, the procedure was repeated, but treatments were alternated. Each rat received each treatment once, with four rats receiving each of the three treatments prior to each session. For each session, half of the vehicle-treated rats received vehicle SC and half received vehicle IP.

PPI, defined as (100- [startle amplitude on prepulse trials/startle amplitude on PULSE trials] × 100), was analyzed using a two-way analysis of variance (ANOVA) with repeated measures on dose. PULSE amplitude was analyzed using a two-way ANOVA with repeated measures on group. Post-hoc comparisons were completed with a Tukey test. Alpha was 0.05.

RESULTS

The effects of clozapine and haloperidol on APO-disrupted PPI are seen in Fig. 1. In rats pretreated with clozapine, ANOVA with repeated measures on pretreatment and treatment revealed a significant effect of APO [$F(1, 62) = 111.35$, $p < 0.0001$], a significant effect of clozapine [$F(5, 62) = 7.51$, $p < 0.0001$] and a significant APO × clozapine interaction [$F(5, 62) = 5.40$, $p < 0.0005$). Post-hoc Tukey tests revealed that the APO-disruptive effects on PPI were significantly decreased by 9 and 12 mg/kg doses of clozapine. In rats pretreated with haloperidol, ANOVA revealed a significant effect of APO [$F(1, 35) = 51.57$, $p < 0.0001$], a significant effect of haloperidol [$F(4, 35) = 18.75$, $p < 0.0001$], and a significant APO × haloperidol interaction [$F(4, 35) = 13.36$, $p < 0.0001$]. Post-hoc Tukey tests revealed that the APO-disruptive effects on PPI were significantly decreased by all doses of haloperidol tested. Thus, the significant disruption of PPI induced by APO was reversed in a dose-dependent manner by both clozapine and haloperidol.

The effects of clozapine and haloperidol on the baseline

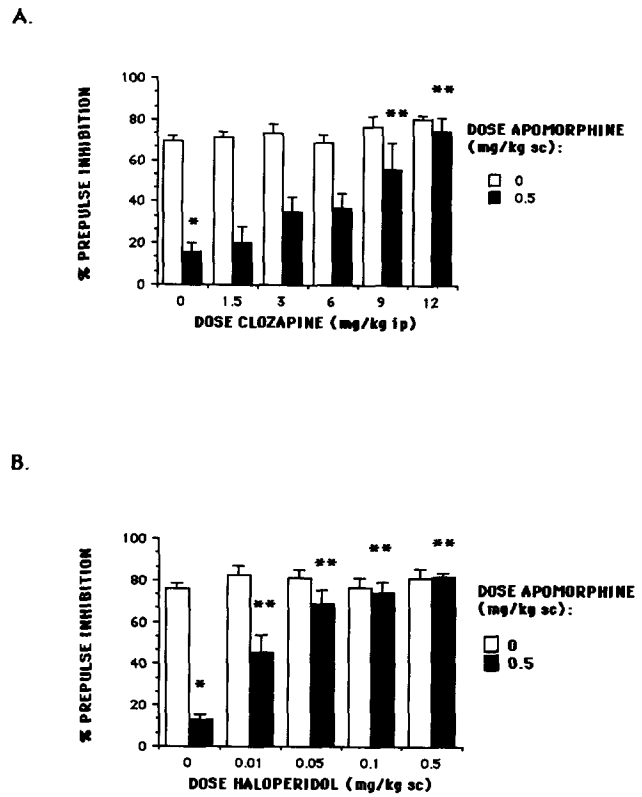

FIG. 1. Effects of clozapine (A) and haloperidol (B) on the APO-disruption of PPI. In each case, APO (0.5 mg/kg SC) caused a significant decrease in PPI that was reversed in a dose-dependent manner by clozapine and haloperidol. *Significant effect of APO by Tukey test after significant main effect of APO by ANOVA; **Significantly greater than 0 mg/kg pretreatment by Tukey test after significant treatment × pretreatment interaction by ANOVA.

PPI induced by weak prepulses are seen in Fig. 2. ANOVA with repeated measures on drug and prepulse intensity revealed a significant effect of prepulse intensity [$F(4, 44) = 38.73, p < 0.0001$], a significant effect of drug [$F(2, 22) = 3.93, p < 0.035$], and no significant intensity × drug interaction ($F < 1$). Independent ANOVAs revealed that PPI was significantly increased by clozapine [$F(1, 11) = 12.71, p < 0.005$]. Although the effect of haloperidol on PPI did not reach statistical significance [$F(1, 11) = 2.69, p < 0.13$], the effects of clozapine and haloperidol did not significantly differ from each other ($F < 1$) . Analysis of PULSE amplitude revealed a significant effect of treatment [$F(2, 11) = 8.64, p < 0.002$]. Post-hoc one-way ANOVAs revealed that, compared to vehicle, clozapine significantly decreased PULSE amplitude [$F(1, 11) = 13.9, p < 0.005$], but haloperidol did not decrease PULSE amplitude ($F < 1$).

DISCUSSION

Since our initial report that deficient PPI in schizophrenic patients is mimicked in rats treated with DA agonists such as APO (18), amphetamine (10) and quinpirole (12), others have reported that antipsychotics restore PPI in APO-treated rats (13) and in schizophrenic patients (24). In the present report, we confirm and extend our previous observation (19) that the APO-disruption of PPI in rats is reversed by both typical and atypical antipsychotics. This effect appears to be specific to the class of antipsychotic drugs and well-correlated with the clinical potency of the antipsychotics (22). Compared to our earlier report, the paradigm used in the present study was designed to more clearly assess this phenomenon, using a single prepulse condition, a shorter startle session, and a measure of PPI that is less succeptible to artifact resulting from drug-induced changes in startle reactivity. With these paradigmatic modifications, the effects of clozapine, like those of other antipsychotics, were more monotonically related to dose. By contrast, the previous findings were supportive of an inverted-U-shaped relationship between dose of clozapine and the restoration of PPI in APO-treated rats. The relative potency of clozapine and haloperidol in the present study (clozapine approximately 100 times less potent) is comparable to previous reports in this animal model (17) and in clinical studies (9).

We also report that clozapine increases "baseline" PPI in a paradigm in which weak prepulses produce submaximal levels of PPI. Thus, by improving PPI, clozapine produced effects on PPI that are opposite to those produced by dopamine agonists. The effects of haloperidol were qualitatively similar to clozapine in this paradigm, but this effect did not reach statistical significance. Though not conclusive, the present results may reflect a superiority of clozapine over haloperidol in improving PPI in rats. Interestingly, clozapine may be more effective than haloperidol in increasing PPI in schizophrenic patients (24). In the present study, clozapine significantly depressed PULSE amplitude, while haloperidol did not. Since PPI was calculated using a percentage score, such a decrease in PULSE amplitude would not be expected to artificially increase the amount of PPI; if anything, a possible "floor" effect might be expected to artificially decrease the observed PPI, which is opposite to what was noted with clozapine.

Further parametric investigations of the circumstances in which antipsychotic-induced enhancements of PPI are observed will help us study putative antipsychotic drugs in a test paradigm that does not require the administration of a

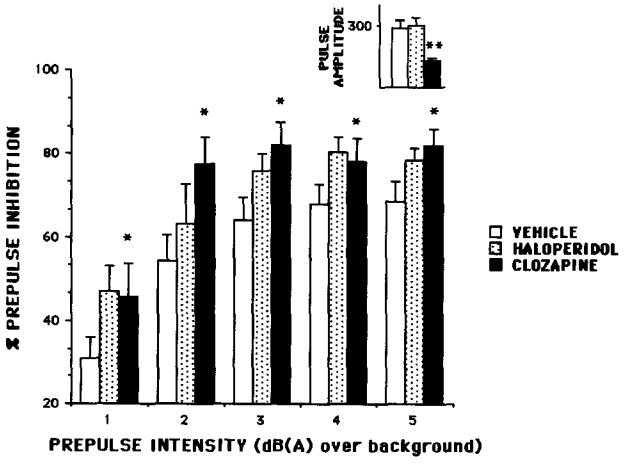

Fig. 2. Effects of clozapine (12 mg/kg IP) and haloperidol (0.1 mg/kg SC) on "baseline" PPI tested using weak (1-5 dB(A) over background) prepulses. Inset figure details effects of clozapine and haloperidol on startle reactivity (amplitude). *Significantly greater than vehicle group by independent ANOVA test after significant effect of treatment by ANOVA; **Significantly lower than vehicle group by one-way ANOVA after significant main effect of treatment by ANOVA.

dopamine agonist. With the exception of the latent inhibition paradigm (23), most animal measures that are relevant to schizophrenia rely on the ability of dopamine agonists to elicit the behavior of interest. In the present study, the antipsychotic clozapine produced a behavioral effect in rats that is opposite to the closely related behavioral deficit observed in schizophrenic patients.

In summary, the present study confirms that clozapine and haloperidol restore PPI in APO-treated rats, suggesting that the APO-disruption of PPI may be a useful predictive model for screening both typical and atypical antipsychotic compounds. Our findings also suggest that the increase in "baseline" PPI might also be a useful measure for assessing antipsychotic potency, but this preliminary observation must be supported by a substantial amount of parametric analyses, as well as controls for the pharmacologic specificity of this effect.

ACKNOWLEDGEMENTS

This work was supported by NIMH Grant MH-42228 (MAG,-NRS), R29 MH48381-01A1 (NRS) and RSDA Grant MH-00188 (MAG). The authors gratefully acknowledge the excellent assistance of Mr. Navid Taaid, Ms. Heidi Hartston, and Ms. Pamela Auerbach. Methodology in this study was identical to that described in previous work by the authors (see 14,15,19). Clozapine was generously provided by Sandoz Pharmaceuticals (Berne, Switzerland). Some of the present findings were reported in abstract form (16).

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