Novel Antipsychotic-Like Effects on Prepulse Inhibition of Startle Produced by a Neurotensin Agonist

DAVID FEIFEL, TAMMI L. REZA, DAVID J. WUSTROW and M. DUFF DAVIS

Department of Psychiatry, University of California, San Diego, La Jolla, California (D.F., T.L.R.), and Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, Michigan (D.J.W., D.D.)

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ABSTRACT

Agonists of the neuropeptide neurotensin have been proposed as potential novel antipsychotics based on their ability to modulate neurotransmission in brain regions associated with schizophrenia. To test this hypothesis, we examined the effects of a neurotensin mimetic with strong metabolic stability in an animal model with strong predictive validity for antipsychotic activity. Subcutaneous injections of PD149163, a reduced amide neurotensin(8–13) mimetic, significantly antagonized the reduction of prepulse inhibition (PPI) of the rat startle reflex produced by amphetamine and by the phencyclidine analog dizocilpine. PD149163 had no significant effect on baseline PPI or on baseline startle amplitude and did not antagonize the reduction of PPI produced by the direct dopamine agonist apomorphine. These findings suggest that PD149163 has novel antipsychotic-like properties that are distinct from known members of both the “typical” and “atypical” families of antipsychotics.

Neurotensin is a neuropeptide that exists in several brain areas that are highly relevant to schizophrenia (Manberg et al., 1982; Hokfelt et al., 1984). The central administration of neurotensin produces effects consistent with the actions of neuroleptics (Ervin et al., 1981; Nemeroff et al., 1983; Kali vas et al., 1984; Jolicoeur et al., 1993), raising the possibility that neurotensin agonists may have use as antipsychotics (Nemeroff et al., 1992). A major barrier to the development of neuropeptides as psychotropic drugs has been their short half-life and poor penetration of the central nervous system after systemic administration. For example, neurotensin(8–13) is the smallest neurotensin fragment with full biological activity (Kanba et al., 1988), but it does not produce observable effects in the central nervous system after systemic administration (Michida et al., 1993).

PD149163 is a reduced amide bond neurotensin(8–13) mimetic with strong affinity for neurotensin receptors (Kᵢ = 31.2 nM in newborn mouse brain membranes) and improved metabolic stability (Wustrow et al., 1995), a factor that promotes central activity after systemic administration (Banks et al., 1994). In this study, we tested the hypothesis that neurotensin agonists may possess antipsychotic properties by examining the effects of systemically administered PD149163 in an animal model of information processing deficits associated with schizophrenia, which is a strongly predictive preclinical screen for antipsychotic activity (Swerdlow et al., 1994a).

The PPI of the acoustic startle reflex is the normal suppression of the startle response when the intense startling sound (“pulse”) is immediately preceded by a much weaker sound (“prepulse”). PPI is an operational measure of sensorimotor gating. Schizophrenic patients have decreased PPI relative to normal control subjects, and this is thought to reflect an impairment in their ability to filter irrelevant sensory stimuli (Braff and Geyer, 1990; Grillon et al., 1992). Similar reductions in PPI are produced in rats by administering psychotomimetic drugs such as the dopamine agonists amphetamine and apomorphine (Mansbach et al., 1988) or the noncompetitive N-methyl-D-aspartate (NMDA) antagonists phencyclidine (PCP) and dizocilpine (MK801) (Mansbach and Geyer, 1989).

All antipsychotics tested are able to antagonize PPI disruption produced by dopamine agonists, whereas PPI disruption produced by NMDA antagonist may be selectively sensitive to antipsychotics with “atypical” features. (Keith et al., 1991; Swerdlow and Geyer, 1993, Swerdlow et al., 1994a; Bakshi et al., 1994). In this study, we tested the effects of systemically injected PD149163 on baseline PPI and PPI reduced by amphetamine, apomorphine, and dizocilpine.

Materials and Methods

Male Sprague-Dawley rats weighing 225 to 250 g on arrival were housed in groups of two and maintained on a 12-h/12-h light/dark schedule (lights on at 7:00 AM), with food and water provided ad libitum. Behavioral testing occurred between 9:00 AM and 3:00 PM. Animals were handled individually within 3 days of arrival and

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ABBREVIATIONS: PPI, prepulse inhibition; ANOVA, analysis of variance; PCP, phencyclidine; NMDA, N-methyl-D-aspartate.
regularly thereafter. All experimental procedures were conducted in accordance with the University of California, San Diego guidelines for animal care and experimentation.

On test days, animals received one of four doses of PD149163 (0, 0.08, 0.25, or 1 mg/kg) administered s.c. and dissolved in 0.9% saline (volume, 1 ml/kg). Immediately after this injection, animals received a second s.c. injection consisting of either d-amphetamine (2 mg/kg), apomorphine (0.5 mg/kg), dizocilpine (0.1 mg/kg), or saline. Ten minutes after this second s.c. injection, animals were placed in separate startle chambers (SR-LAB; San Diego Instruments, San Diego, CA). Startle chambers consist of a Plexiglas cylinder 8.2 cm in diameter resting on a 12.5 × 25.5-cm Plexiglas frame within a ventilated enclosure housed in a sound-attenuated room exposed to a 70-dB background noise. After a 5-min acclimation period, acoustic stimuli were presented via a speaker mounted 24 cm above the animal. Acoustic stimuli consisted of a 120-dB pulse by itself (pulse alone) or a 120-dB pulse preceded, 100 ms, by prepulses of 3, 5, and 10 dB above background noise. There was an average of 15 s between stimuli. A piezoelectric accelerometer mounted below the Plexiglas frame detected and transduced the motion within the cylinder. Startle amplitude was defined as the degree of motion detected by this accelerometer. Each rat was tested on four separate occasions separated by 7 nontest days. On each test day, the dose of PD149163 that each rat received was kept constant, but the specific psychotomimetic agent was alternated across test days in a counterbalanced fashion.

Prepulse inhibition was calculated as a percentage of the pulse-alone startle amplitude using the following formula: [1 – (startle amplitude after prepulse-pulse pair/startle amplitude after pulse only) × 100]. Analysis of data was then carried out using a three-factor (PD149163 dose × psychotomimetic treatment × prepulse intensity), repeated measures analysis of variance (ANOVA). Significant factor results from the ANOVA were followed up with separate one-way ANOVAs for each psychotomimetic agent and then, where indicated, with individual group mean comparisons using post hoc t tests adjusted for multiple comparisons using the Bonferroni method.

Results

There was a significant effect of psychotomimetic drug [F(3, 87) = 45.72, P < .001] as each of the active psychotomimetics reduced PPI relative to saline (Fig. 1). Prepulse intensity had a significant effect [F(2, 58) = 66.7, P < .001], with more intense prepulses producing greater PPI (Table 1). The effect of PD149163 dose on PPI was significant [F(3, 29) = 3.11, P < .05], and the PD149163 dose × psychotomimetic drug interaction approached significance [F(9, 87) = 1.73, P = 0.094; Fig. 1], as did the psychotomimetic × prepulse intensity × PD149163 dose interaction [F(18,174) = 1.63, P = 0.054]. Separate, one-way ANOVAs revealed a significant effect of PD149163 dose in animals treated with amphetamine [F(3, 29) = 5.17, P = 0.006] and dizocilpine [F(3, 29) = 3.45, P = 0.029] but not with saline [F(3, 29) = 0.27, P = 0.84] or apomorphine [F(3, 29) = 0.57, P = 0.64]. None of the PD149163 doses tested had a significant effect on PPI in saline-treated rats. PD149163 dose-dependently reversed amphetamine-reduced PPI, fully blocking its effect at the highest dose tested (1.0 mg/kg). In contrast, none of the doses of PD149163 treated had a significant effect on apomorphine-reduced PPI. In rats given dizocilpine, PD149163 dose-dependently increased PPI, with the highest doses (0.25 and 1.0 mg/kg) reaching statistical significance.

There was a significant main effect of psychotomimetics on pulse-alone startle amplitude [F(3, 87) = 24.04, P < .001], with a tendency of dizocilpine to increase it relative to saline. PD149163 had no significant overall effect on pulse-alone startle amplitude [F(3, 29) = 1.72, P = .18], although separate, one-way ANOVAs revealed a significant effect of PD149163 dose in animals given saline injections [F(3, 29) = 3.61, P < .05], with the highest dose producing a statistically significant reduction in pulse-alone startle amplitude. Dizocilpine [F(3, 29) = 0.89, NS], amphetamine [F(3, 29) = 2.24, NS], and apomorphine [F(3, 29) = 0.37, NS] did not produce significant main effects on pulse-alone startle. Pulse-alone startle amplitude was decreased in animals receiving the highest dose of PD149163 across all psychomimetic conditions, but this reduction was statistically significant only in animals given saline injections (Fig. 2). There was no significant interaction effect on startle amplitude between PD149163 dose and the psychotomimetics [F(9, 87) = 0.38, P = .39].

![Fig. 1. The effects of PD149163 on prepulse inhibition of the startle reflex in rats given s.c. injections of saline or the direct dopamine agonist apomorphine (0.5 mg/kg), the indirect dopamine agonist amphetamine (2 mg/kg), and the glutamate antagonist dizocilpine (0.1 mg/kg). Significant differences from the rats treated with same dose of PD149163 but no active psychotomimetic (saline) are represented by *; P < .05; **, P < .01; and *** P < .001. Significant differences from the corresponding 0 mg/kg PD149163 condition are represented by the symbol (P < .05). n = 7–9 in all treatment groups.](https://example.com/Fig1.png)
The ability of PD149163 to dose-dependently block amphetamine-reduced PPI is consistent with the antidopamine actions attributed to neurotensin in previous studies (Ervin et al., 1981; Nemeroff et al., 1983; Kalivas et al., 1984; Jolicoeur et al., 1993). Both “typical” antipsychotics, such as haloperidol, and “atypical” antipsychotics, such as clozapine, reverse PPI reduced by dopamine agonists (Swerdlow and Geyer, 1993, Swerdlow et al., 1998). Wan et al. (1995), found that 6-cyano-7-nitroquinolin-2,3-dione, a non-NMDA antagonist, blocked disruption of PPI produced by amphetamine but not by quinprolole, a nonselective dopamine receptor agonist (Swerdlow et al., 1991) and oxytocin (Feifel et al., 1998). PD149163 can form such a complex with dopamine released from synaptic terminals by amphetamine, but not with apomorphine. However, it is also possible that PD149163 interferes with unidentified postsynaptic aspects of dopamine transmission that are less potently activated by apomorphine than by endogenous dopamine, whose release is stimulated by amphetamine. A similar selective ability to block amphetamine, but not apomorphine, disruption of PPI has been produced with an opiate antagonist (Swerdlow et al., 1991) and oxytocin (Feifel et al., 1998). Wan et al. (1995), found that 6-cyano-7-nitroquinolin-2,3-dione, a non-NMDA antagonist, blocked disruption of PPI produced by amphetamine but not by quinprolole, a direct D2/D3 agonist. This suggests that certain drugs may modulate PPI by way of a presynaptic mechanism of action that interferes with dopamine release from synaptic terminals. The administration of neurotensin into the nucleus accum-
bens has been shown to antagonize and potentiate the disruption of PPI produced by dopamine agonists at low doses and high doses, respectively (Feifel et al., 1997b,a). It has been suggested that both antidopamine and prodopamine mechanisms of neurotensin modulation may exist (Feifel et al., 1997b). The monophasic antagonism by PD149163 of the effect of amphetamine may be due to selective activation of the putative antidopamine mechanism within the nucleus accumbens.

The mechanism by which NMDA antagonists disrupt PPI is not known. PCP and dizocilpine stimulate the release of dopamine from nerve terminals in the nucleus accumbens (Schmidt and Fadayel, 1996). The ability of PD149163 to antagonize dizocilpine-induced disruption of PPI may be due to inhibition of this dopamine release or the formation of complex with dopamine. This would be consistent with the antagonism by PD149163 of the PPI effects of amphetamine.

It is of interest that the ability to antagonize PCP- or dizocilpine-induced disruption of PPI is not consistently exhibited by all antipsychotic drugs. Haloperidol has been found not to produce this effect (Keith et al., 1991; Johansson et al., 1994; Swerdlow et al., 1996), whereas atypical antipsychotics such as clozapine, risperidone, olanzapine, and zipatiene have been found by some authors (Bakshi et al., 1994; Bakshi and Geyer, 1995; Varty and Higgens, 1995; Swerdlow et al., 1996) but not others (Hoffman et al., 1993; Johansson et al., 1994; Varty and Higgens, 1995) to have this ability. The ability of PD149163 to antagonize dizocilpine-induced disruption of PPI thus further distinguishes its antipsychotic-like effect from that of haloperidol. This finding, together with its ability to block amphetamine- and not apomorphine-induced changes in PPI, suggests that PD149163 has novel antipsychotic-like characteristics.

The fact that PD149163 had no significant effect on baseline PPI suggests that its effects on amphetamine- and dizocilpine-reduced PPI are not due to a nonspecific ability to enhance PPI. Similarly, PD149163 had no statistically significant effect on startle amplitude in rats, which also suggests that its effects on PPI are specific and not due to general motor effects or specific effects on the primary startle mechanism. High doses of PD149163 produced a tendency to decrease startle amplitude. This effect on startle amplitude is not likely to have significant contributed to the effect of PD149163 on PPI because the effects of PD149163 on PPI in rats were found to be statistically significant.

In summary, these results indicate that PD149163 affects psychomimetic-induced deficits in PPI in a manner that is similar to the effects of atypical antipsychotics in some respects but also differs from members of both the typical and atypical antipsychotic families in important ways. These findings support the theory that neurotensin agonists designed to have central activity after systemic administration hold significant promise as potential novel antipsychotics.

References
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