Effects of LU-111995 in Three Models of Disrupted Prepulse Inhibition in Rats

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ABSTRACT

LU-111995 is a novel antipsychotic drug in clinical development. It has a clozapine-like receptor profile and affinities for dopamine D4 and 5-hydroxytryptamine2A receptors. The effects of LU-111995 were examined in three models of disrupted prepulse inhibition (PPI) in rats. The first model tested the hypothesis that LU-111995 would normalize the deficit in PPI exhibited by rats treated with the dopamine agonist apomorphine. LU-111995 significantly reduced the effect of apomorphine on PPI but also slightly increased PPI by itself. Thus, the increases in PPI were not specific to the animals treated with apomorphine but reflected an effect of LU-111995 on PPI. LU-111995 also attenuated the apomorphine-induced increase in startle reactivity. The second model tested the hypothesis that LU-111995 would normalize the deficit in PPI exhibited by rats treated with the psychotomimetic phencyclidine (PCP). LU-111995 significantly blocked the PCP-induced increase in startle reactivity but did not alter the PPI-disruptive effects of PCP. The third model tested the hypothesis that LU-111995 would normalize the deficit in PPI exhibited by isolation-reared rats treated as adults. Isolation rearing of rats produced deficits in PPI. LU-111995 reversed the isolation rearing-induced deficit in PPI without having any significant effect on PPI in socially reared rats. In summary, LU-111995 exhibits potential antipsychotic-like activity in two models of disrupted PPI. It remains to be elucidated whether its effects on PPI can be attributed to a blockade of single dopamine and 5-hydroxytryptamine receptor subtypes, especially D4 and 5-hydroxytryptamine2A, or a combination of both.

LU-111995 (Knoll AG, Ludwigshafen, Germany) is a candidate antipsychotic, chemically distinct from clozapine but with a similar mixed receptor profile. In radioligand binding studies, it displayed the highest affinities for human dopamine (DA) D4, human 5-hydroxytryptamine (serotonin; 5-HT2A) receptors, followed by histamine H1 and α1-adrenergic receptors. Like clozapine, LU-111995 had a higher affinity for D4 and 5-HT2A receptors than for D2 receptors but did not bind to muscarinic receptors. LU-111995 did not induce catalepsy, increase plasma prolactin, or increase DA release in the rat brain, all features that suggest low D2 receptor occupancy (data on file, Knoll AG; Steiner et al., 1998). In preliminary clinical studies, LU-111995 exhibited antipsychotic effects (Steiner et al., 1998). It is unknown whether its therapeutic effect is mediated by antagonism at D4 or 5-HT2A receptors, or a combination of both.

The present study explored the effects of LU-111995 in three rodent models relevant to schizophrenia: 1) apomorphine-induced deficits in prepulse inhibition (PPI) of startle, 2) phencyclidine (PCP)-induced PPI deficits, and 3) isolation rearing-induced PPI deficits. PPI is a multimodal, cross-species phenomenon used as an operational measure of sensorimotor gating (Ison and Hoffman, 1983; Braff and Geyer, 1990). Sensory overstimulation and accompanying cognitive fragmentation may be features of schizophrenia (McGhie and Chapman, 1961). Deficits in sensorimotor gating, as assessed by PPI, are observed in schizophrenic patients (Braff et al., 1978, 1992; Grillon et al., 1992; Bolino et al., 1994) and in nonmedicated schizotypal subjects (Cadenhead et al., 1993); some evidence indicates that antipsychotics reverse PPI deficits in schizophrenic patients (Weike et al., 1996).

In rats, PPI is reduced by direct (apomorphine) and indirect (amphetamine) DA agonists (Mansbach et al., 1988; Peng et al., 1990); these effects are reversed by DA D2-like receptor antagonists (Mansbach et al., 1988; Swerdlow et al., 1991). The apomorphine disruption of PPI is also reversed by the atypical antipsychotic clozapine (Swerdlow et al., 1991; Swerdlow and Geyer, 1993) and the putative atypical anti-

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ABBREVIATIONS: DA, dopamine; PPI, prepulse inhibition; 5-HT2A, 5-hydroxytryptamine (serotonin); PCP, phencyclidine; NMDA, N-methyl-D-aspartate.
psychotic quetiapine (Swerdlow et al., 1994). The putative D₄ antagonists L-745,870, U-101,387 and CP-293,019 also restore PPI in apomorphine-treated rats (Mansbach et al., 1998; Zorn et al., 1996), although Bristow et al. (1997) reported that the D₄ antagonist L-745,870 failed to reverse the apomorphine effects on PPI. Corbin and Heffner (1997) recently reported that among four “selective” D₄ antagonists, two potently reversed the apomorphine effect, whereas two others were ineffective. The present study examined whether the atypical antipsychotic LU-111995, with its high D₄ affinity, reverses apomorphine effects on PPI.

In rats, PPI is reduced by the psychotomimetic noncompetitive N-methyl-d-aspartate (NMDA) antagonists PCP and dizocilpine (Mansbach and Geyer, 1989). PCP is a powerful psychotogen that in healthy humans causes symptoms that resemble both the positive and negative symptomatology of schizophrenia, and acutely exacerbates existing behavioral and cognitive impairments in schizophrenic patients (Lahti et al., 1995; Malhotra et al., 1996). Unlike the effects of DA agonists on PPI, those of PCP or dizocilpine are not reversed by high-potency typical antipsychotics such as haloperidol (Geyer et al., 1990; Keit et al., 1991), but are reversed by atypical antipsychotics such as clozapine (Bakshi et al., 1994), olanzapine (Bakshi and Geyer, 1995), and quetiapine (Swerdlow et al., 1996), and by the putative antipsychotic and 5-HT₂A antagonist M100907 (Varty et al., 1999). Hence, the PCP-induced disruption of PPI might be useful for identifying compounds with atypical antipsychotic potential. Therefore, an additional aim of this study was to test LU-111995 on the PCP-induced disruption of PPI.

Although pharmacological approaches that alter PPI help to identify relevant neural substrates, they do not assess environmental or developmental contributions to PPI deficits. Because schizophrenia appears to be in part a neurodevelopmental disorder (e.g., Weinberger, 1987), it is important to incorporate the developmental perspective into animal models of sensorimotor-gating deficits in schizophrenia. One such approach is isolation rearing of rats (Geyer et al., 1993). Rats reared in single housing from weaning through adulthood exhibit deficient PPI compared with socially reared counterparts (Geyer et al., 1993; Wilkinson et al., 1994; Bristow et al., 1995; Varty and Higgins, 1995). Multiple neurochemical (e.g., altered DA and serotonin levels within limbic regions) and behavioral (e.g., hyperactivity and hypersensitivity to DA and serotonin agonists) abnormalities are produced by isolation rearing in rats (Bakshi et al., 1996; Hall et al., 1998). Isolation rearing-induced deficits in PPI are reversed by treatment with either typical or atypical antipsychotics (Geyer et al., 1993; Varty and Higgins, 1995; Bakshi et al., 1998), including the putative antipsychotic and 5-HT₂A antagonist M100907 (Geyer et al., 1998). The present studies examined the effect of LU-111995 on isolation-induced PPI deficits in rats.

**Materials and Methods**

**Animals**

**Apomorphine and PCP Test Paradigms.** Experimentally naive male Sprague-Dawley rats (Harlan, San Diego, CA) weighing 300 to 400 g were used. Rats were housed two per cage on a reversed 12-h light/dark cycle (lights off 7:00 AM–7:00 PM) in a temperature- and light-controlled animal facility. Rats had free access to standard laboratory diet (Harlan Teklad) and filter water.

**Isolation Rearing Test Paradigm.** Male Sprague-Dawley rats weighing 35 to 49 g were obtained at 20 to 21 days of age (Harlan, San Diego, CA). Rats were separated randomly into two groups and housed for the entire study in the same temperature- and light-controlled animal room on a reversed light/dark cycle. Isolation-reared rats were housed individually in plastic cages (44 × 24 × 21 cm high), whereas socially reared rats were housed in groups of two or three rats per cage in the same size cages. All rats could hear, see, and smell other rats. Food (Harlan Teklad) and water were freely available throughout the study. Neither group was handled except during behavioral testing, which occurred between 9:00 AM and 5:00 PM. Experiments were carried out after 8 weeks of isolated or social rearing. Health checks were conducted daily via visual inspection.

**Startle Apparatus**

Four SR-LAB (San Diego Instruments, San Diego, CA) test stations were used to elicit and monitor startle responses. Each lighted and ventilated chamber contained a stabilimeter comprised of an 8.2-cm-diameter Plexiglas cylinder mounted on a Plexiglas base, as described previously (Geyer and Swerdlow, 1998). A speaker mounted 24 cm above the rat provided the background noise, prepulse stimuli, and startle stimuli, which were controlled by the SR-LAB system. Startle responses were transduced by a piezoelectric accelerometer mounted below the cylinder, digitized (0–4095), rectified, and recorded as 100 1-ms readings, starting at the onset of each startle stimulus. The average of these 100 readings was used as the dependent measure. Calibration procedures were performed periodically to ensure consistent levels of loudspeaker performance and stabilimeter sensitivity. Sound levels were measured as described previously (Mansbach et al., 1988; Geyer and Swerdlow, 1998) using the db(A) scale with a calibrated Quest Sound Level Meter.

**Drugs**

Apomorphine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in a 0.1% ascorbic acid vehicle. PCP hydrochloride (Research Biochemicals, Natick, MA) was dissolved in 0.9% saline. Injections of either saline, vehicle, apomorphine, or PCP were administered at 1.0 ml/kg volume by the s.c. route. With the exception of experiment 3, LU-111995 [(+)-4S,5R,6S]-exo-3-[4-fluorophenyl]-3-azabicyclo[3.2.0]heptane-3-yl-(4H,3H-quinoxaline-2,4-dione fumarate] (Knoll AG, Ludwigshafen, Germany) was dissolved in acidified distilled water and kept at body temperature before injection. Injections of either vehicle or LU-111995 were administered at 1.0 ml/kg volume for doses up to 3.0 mg/kg (free base) and 2.0 ml/kg for the 10.0 mg/kg (free base) dose i.p. In experiment 3, LU-111995 was dissolved in an aqueous solution of tartaric acid and administered i.p., at a dose of 10.0 mg/kg (free base) in a 3.0 ml/kg volume.

**Baseline Matching Procedure**

Within each experiment, with the exception of the isolation rearing study, all rats were tested initially in a brief test session to define matched groups. The baseline test session consisted of 15 trials in which the response to a 40-ms burst of 120 db[A] noise was measured, interspersed with five prepulse + pulse trials (prepulses 12 db above background noise level). The average response of each rat to the 120-db noise bursts across the 15 trials was used to define groups matched for both the mean and range. Matching was done separately for each stabilimeter chamber, each rat being retested in the same chamber.

**PPI Test Procedures**

**Apomorphine and PCP Test Sessions.** Rats were placed into the startle apparatus and allowed a 5-min acclimation period, which included the administration of 65-db background noise. In both the
apomorphine and PCP test paradigms, the startle session consisted of two components, both using 120-db startle pulses (40 ms in duration). Within each component, three or four different intensities of acoustic prepulse stimuli (3, 6, and 12 or 1, 2, 3, or 4 db above background) were used, each prepulse being 20 ms in duration. The first component tested 3-, 6-, and 12-db prepulses to match the range of intensities typically used in studies testing antipsychotic-induced reversals of experimentally disrupted PPI. The two higher prepulse intensities are generally the most reliable for demonstrations of disruptions in PPI produced by apomorphine or PCP (Geyer et al., 1990; Bakshi and Geyer, 1995). Lower prepulse intensities were investigated in the second component of the session to determine whether the putative antipsychotic LU-111995 might improve PPI by itself or by clozapine (Swerdlow and Geyer, 1993). The first component began with four pulse-alone trials preceding a pseudorandom sequence of trials in which the rats received pulse alone (16 trials), prepulse + pulse (10 trials at each prepulse intensity), or no-stimulus trials between each stimulus trial. The initial component ended with four pulse-alone trials. Habituation was defined as the first pulse-alone responses (block 1), the average of the next pulse-alone trials divided into two equal blocks (blocks 2 and 3), and the last pulse-alone responses (block 4). The second component consisted of pulse alone (eight trials) and prepulse + pulse trials (five trials at each prepulse intensity) presented in a pseudorandom sequence as well as no-stimulus trials between each stimulus trial. The time from onset of the prepulse to onset of the pulse was always 100 ms. Because no appreciable group differences were observed on the no-stimulus trials, these data are not reported. In all experiments, the variable interval between trials averaged 7.5 s, including the no-stimulus trials. Thus, the intervals between startle trials averaged 15 s.

**Isolation Rearing Test Session.** The test session utilized in the isolation rearing experiment was nearly identical with the first component of the session described above and included: 1) pulse-alone trials in which a 40-ms, 120-db broadband noise burst was presented; 2) prepulse + pulse trials in which the onset of a 20-ms broadband noise preceded the onset of the 120-db pulse by 100 ms (prepulse intensities that were 3, 6, and 12 db above the background noise were used); and 3) no-stimulus trials in which no stimuli were presented. The pulse-alone trials were presented 14 times; each of the prepulse + pulse trials was presented 10 times; the no-stimulus trials were presented 8 times. All trials were presented in a pseudorandom order, with an average of 15 s separating consecutive trials. Four pulse-alone trials were presented at the beginning and the end of the test session (for a total of 60 trials), but were not used in the calculation of PPI values. A background noise of 65 db was presented for 5 min after the rats were placed in the startle chambers (the acclimation period) and continued throughout the entire session. Habituation was defined as the first pulse-alone responses (block 1), the average of the next pulse-alone trials divided into two equal blocks (blocks 2 and 3), and the last pulse-alone responses (block 4). This test session has been used previously to detect restorations of PPI in apomorphine-treated rats. Each rat was tested twice at an interval of 1 week. Half the rats in each group received s.c. injections of vehicle or apomorphine in the first test, with each rat receiving the opposite treatment in the second test 1 week later. Each rat received the same pretreatment before each test. Thus, pretreatment was a between-subject factor and treatment was a within-subject factor. Experiments 2 and 3 were run in the same manner as experiment 1 with the exception that a higher dose of LU-111995 (10 mg/kg) was used. In experiment 3, the dose of LU-111995 was 10 mg/kg, and two doses of apomorphine (0, 0.1, and 0.5 mg/kg) were examined over a 3-week period.

**Experiment 4: PCP and LU-111995.** Experiment 4 examined startle responding and PPI in rats pretreated with either vehicle or low doses of LU-111995 (0.3, 1.0, and 3.0 mg/kg) and treated 30 min later with either vehicle or 0.5 mg/kg apomorphine. Animals were then placed directly into the startle test chambers. This study tested the hypothesis that the D₄/5-HT₂A antagonist LU-111995 would normalize the deficit in PPI exhibited by apomorphine-treated rats. Each rat was tested twice at an interval of 1 week. Half the rats in each group received s.c. injections of vehicle or apomorphine in the first test, with each rat receiving the opposite treatment in the second test 1 week later. Each rat received the same pretreatment before each test. Thus, pretreatment was a between-subject factor and treatment was a within-subject factor. Experiments 4 and 5 were run in the same manner as experiment 1 with the exception that a higher dose of LU-111995 (10 mg/kg) was used. In experiment 5, the dose of LU-111995 was 10 mg/kg, and two doses of apomorphine (0.1, 0.5 mg/kg) were examined over a 3-week period.

**Experiment 5: LU-111995 and Isolation Rearing.** Experiment 5 compared 26 isolation-reared rats with 30 socially reared rats by using measures of startle responding. All rats were tested after 8 weeks of the appropriate housing. This study tested the hypothesis that LU-111995 would normalize the deficit in PPI exhibited by isolation-reared rats. Each rat was tested twice in the startle session. Half the rats in each housing group received i.p. injections of vehicle or 10.0 mg/kg LU-111995 30 min before the first test, with each rat receiving the opposite treatment in the second test 1 week later.

**Results**

**Experiment 1: 0.5 mg/kg Apomorphine and 0.3, 1.0, or 3.0 mg/kg LU-111995.** As shown in Fig. 1A, LA-111995 (0.3, 1.0, or 3.0 mg/kg) had no effect on startle reactivity (F₁,2₈ = 1.4, N.S.), and there was no interaction with block (F₈,₈₄ = 1.0, N.S.). There was a main effect of apomorphine treatment to elevate startle reactivity (F₁,₁₈ = 34.2, p < 0.01) and there was a significant block × treatment interaction (F₈,₈₄ = 3.8, p < 0.02; Fig. 1A, top). Rats pretreated with LU-111995 exhibited a significant decrease in this apomorphine effect (block × drug × pretreatment interaction: F₈,₈₄ = 2.1, p < 0.05; Fig. 1A, bottom).

As expected, rats administered apomorphine exhibited a
deficit in PPI (Fig. 1B). The mixed-design ANOVA on %PPI demonstrated a significant main effect of apomorphine treatment ($F_{1,28} = 207.6$, $p < .01$), no effect of LU-111995 pretreatment ($F_{3,28} = .81$, N.S.), a drug $\times$ prepulse interaction ($F_{2,56} = 35.3$, $p < .01$), and no other significant interactions. Thus, the deficit in %PPI exhibited by apomorphine-treated rats was not altered significantly by low doses of LU-111995 in the first component of the session (3-, 6-, or 12-db prepulse intensities). It should be noted that in this and the subsequent experiments, the drug effects were quite similar at both 6- and 12-db prepulse intensities, although for clarity only the results from the 6-db condition are displayed in the figures. There was neither an effect of LU-111995 by itself on PPI nor any significant interaction between LU-111995 and apomorphine effects in the second component of the session (1-, 2-, 3-, or 4-db prepulse intensities). Although the mean values of %PPI after the combination of LU-111995 and apomorphine were larger than the values after apomorphine by itself, the pretreatment $\times$ treatment interaction was not significant ($F_{3,42} = 4.92$, $p < .01$, with no pretreatment $\times$ treatment interactions; data not shown).

**Experiment 2: 0.5 mg/kg Apomorphine and 10 mg/kg LU-111995.** As in experiment 1, rats administered apomorphine showed an increase in startle magnitude when compared with controls ($F_{1,14} = 7.54$, $p < .02$; Fig. 2A, top). The 10.0 mg/kg dose of LU-111995 failed to alter startle by itself ($F_{1,14} = .92$, N.S.) and had no effect on the apomorphine-induced increase in startle reactivity ($F_{1,14} = .01$, N.S.; Fig. 2A, bottom).

Pretreatment with 10 mg/kg LU-111995 significantly increased %PPI when compared with vehicle controls ($F_{1,14} = 10.6$, $p < .01$), whereas treatment with apomorphine disrupted PPI ($F_{1,14} = .30.7$, $p < .01$). Although the mean values of %PPI after the combination of LU-111995 and apomorphine were larger than the values after apomorphine by itself, the pretreatment $\times$ treatment interaction was not significant ($F_{1,14} = .24$, N.S.) and the triple interaction ($F_{2,28} = .79$, N.S.) was also not significant (Fig. 2B). In the second component of the test session, 10.0 mg/kg LU-111995 again failed to increase PPI by itself and also failed to block the disruption of PPI caused by apomorphine (treatment $\times$ in- tenseity interaction $F_{3,42} = 4.92$, $p < .01$, with no pretreat- ment $\times$ treatment interactions; data not shown).

**Experiment 3: 0.1 or 0.5 mg/kg Apomorphine and 10 mg/kg LU-111995.** In experiment 3, the effects of two doses of apomorphine (0.1 and 0.5 mg/kg) were assessed. Apomorphine-treated rats again showed an increase in startle magnitude ($F_{2,76} = 12.1$, $p < .01$), with a treatment by block interaction ($F_{3,228} = 2.4$, $p < .05$; Fig. 3A, top). Rats were pretreated with either vehicle or a 10.0 mg/kg dose of LU-111995, which yielded a significant main effect of pretreatment ($F_{1,38} = 6.5$, $p < .05$). As in experiment 1, the high dose
of LU-111995 prevented the apomorphine-induced increase in startle reactivity (Fig. 3A, bottom) (block by pretreatment by treatment interaction $F_{6,228} = 2.2, p < .05$).

Pretreatment with 10 mg/kg LU-111995 again significantly increased %PPI when compared with vehicle controls ($F_{1,38} = 11.0, p < .005$), whereas apomorphine again disrupted PPI ($F_{2,76} = 89.4, p < .01$). In contrast to experiment 2, 10 mg/kg LU-111995 pretreatment reduced the apomorphine-induced deficit in %PPI at both the 6- and 12-db intensities (intensity $\times$ pretreatment $\times$ treatment interaction: $F_{4,152} = 4.1, p < .01$ (6 db, Fig. 3B; 12 db, data not shown)). In the second component of the test session, 10.0 mg/kg LU-111995 failed to alter either PPI by itself or the effect of apomorphine on PPI (main effect of apomorphine treatment $F_{2,76} = 20.7, p < .01$; treatment $\times$ intensity interaction $F_{6,228} = 4.7, p < .01$, with no pretreatment $\times$ treatment interactions) (data not shown).

Experiment 4: 1.5 mg/kg PCP and 3 or 10 mg/kg LU-111995. As illustrated in Fig. 4A, rats treated with either 3.0 or 10.0 mg/kg LU-111995 did not differ significantly from their corresponding controls in either startle reactivity or habituation ($F_{2,66} = 2.2, N.S.$ and $F_{2,198} = <1.0, N.S.$ respectively). PCP-treated rats (1.5 mg/kg) exhibited a significant increase in startle response as indicated by a significant main effect ($F_{1,66} = 5.2, p < .05$) and a significant block $\times$ treatment interaction ($F_{3,198} = 3.4, p < .05$). When pretreated with the high dose of LU-111995, rats administered PCP failed to show PCP-induced increased startle at blocks 2, 3, and 4 (Tukey’s post hoc $p < .05$).

As expected, PCP disrupted PPI, especially with 3- or 6-db prepulses (main effect $F_{2,66} = 40.0, p < .01$). However, neither the main effect of pretreatment ($F_{1,66} = .2, N.S.$) nor the pretreatment $\times$ treatment interaction ($F_{2,66} = .6, N.S.$) was significant (Fig. 4B). A similar pattern of results was found in the second component, with a main effect of PCP treatment ($F_{1,66} = 41.3, p < .01$), no main effect of pretreatment ($F_{2,66} = .5, N.S.$), and no pretreatment $\times$ treatment interaction ($F_{2,66} = .6, N.S.$; data not shown). Thus, unlike startle reactivity, neither dose of LU-111995 altered the PCP-induced disruption of PPI significantly in either component of the session.

Experiment 5: 10 mg/kg LU-111995 and Isolation Rearing. As depicted in Fig. 5A, there was no main housing effect on startle reactivity ($F_{1,54} = 1.5, N.S.$). In contrast, isolation-reared rats tended to show less habituation of startle than did socially reared rats (housing $\times$ block interaction $F_{3,162} = 2.4, p = .07$). LU-111995 (10.0 mg/kg) significantly decreased startle reactivity in both the socially reared and
isolation-reared rats, especially in the early part of the session (Fig. 5A; main effect treatment $F_{1,54} = 24.7$, $p < .01$; treatment $\times$ block interaction $F_{3,162} = 4.4$, $p < .01$).

As expected, isolation-reared rats exhibited a disruption in %PPI as compared with socially reared rats ($F_{1,54} = 5.8$, $p < .02$). Treatment with LU-111995 yielded a main effect on %PPI ($F_{1,54} = 5.6$, $p < .05$). As predicted, it appeared that LU-111995 altered the disruption of %PPI observed in isolation-reared rats (treatment $\times$ housing interaction ($F_{1,54} = 3.61$, $p = .06$; Fig. 5B). Based on the a priori hypothesis that LU-111995 would reverse the isolation rearing-induced deficit, Newman-Keuls pair-wise comparisons were performed. Isolation-reared rats given vehicle had significantly less PPI than either the socially reared vehicle controls or either of the groups administered LU-111995 at the 6-dB intensity. At the other two intensities, 3-dB and 12-dB, the isolation-reared group treated with vehicle had significantly less PPI than either of the LU-111995 groups but failed to be significantly different from the socially reared vehicle controls.

**Discussion**

The present studies revealed that the putative antipsychotic LU-111995 was somewhat effective in reversing the deficit in PPI produced by apomorphine, ineffective in altering PPI deficits produced by PCP, and was effective in reversing the deficits in PPI produced by isolation rearing in rats. Thus, LU-111995 exhibited a profile of activity in rat models of the sensorimotor gating deficits in schizophrenia that resembled that of typical antipsychotic drugs.

By itself, LU-111995 had inconsistent effects on startle reactivity and PPI. Startle reactivity was reduced slightly by LU-111995 only by the highest dose tested and only in the initial trial block in one of the several experiments (Fig. 5A). The lower doses of LU-111995 also failed to have any significant effects on PPI, even in the second component of the test sessions in which weak prepulses were used to maximize the likelihood of detecting LU-111995-induced increases in PPI. By contrast, the highest dose of LU-111995 (10 mg/kg) increased PPI in the first component of the session in two of the four relevant experiments, although it still had no similar effects in the second component of the session. This PPI-increasing effect of LU-111995 is similar to that produced by the atypical antipsychotic clozapine (Swerdlow and Geyer, 1993). Whereas other typical and atypical compounds have produced trends toward similar effects in Sprague-Dawley rats, such increases in normal levels of PPI in rats are generally small and unreliable (Mansbach et al., 1988; Swerdlow et al., 1991).
The first three experiments assessed the effects of LU-111995 in the dopaminergic model in which PPI is disrupted by the direct DA agonist apomorphine. The highest dose of LU-111995 (10 mg/kg) significantly reduced the effect of apomorphine on PPI elicited by both 6- and 12-db prepulses in experiment 3, but did not have such clear effects in experiment 2. It is important to reiterate, however, that this dose of LU-111995 also slightly, but significantly, increased PPI by itself in both experiments 2 and 3. Hence, although the results are consistent with an LU-111995-induced reversal of the effects of apomorphine, the increases in PPI produced by LU-111995 were not specific to the rats treated with apomorphine. In addition to the consistent disruption of PPI produced by apomorphine in these experiments, apomorphine also increased startle reactivity. In experiments 1 and 3, but not in experiment 2, LU-111995 tended to reverse both behavioral effects of apomorphine, but did not do so consistently.

Experiment 4 assessed the ability of 3 or 10 mg/kg LU-111995 to block the effects of the noncompetitive NMDA antagonist and psychotomimetic PCP on startle reactivity and PPI in rats. We found that 10 but not 3 mg/kg LU-111995 significantly blocked the startle reactivity-increasing effect of PCP (although having little effect by itself in control rats), but did not block the PPI-disruptive effects of PCP. Hence, it appears that the drug was biologically active at 10 mg/kg but did not mimic the antagonistic effects of clozapine, olanzapine, quetiapine, and M100907 on NMDA antagonist-induced disruptions of PPI (Bakshi et al., 1994; Bakshi and Geyer, 1995; Swerdlow et al., 1996; Varty et al., 1999). Insofar as all of these compounds exhibit antagonist actions at 5-HT2A receptors, it remains unclear why LU-111995, which is also an antagonist at 5-HT2A receptors (Steiner et al., 1998), did not similarly reduce the PPI-disruptive effects of PCP. Given that other mixed 5-HT2 antagonists, such as ketanserin and risperidone, fail to mimic the effects of the selective 5-HT2A antagonist M100907 in this paradigm (Bakshi et al., 1994; Varty et al., 1999), it appears that complex interactions may influence the effects of multireceptor antagonists in the PCP model of the sensorimotor gating deficits in schizophrenia.

Overall, with the possible exception of experiment 2, the effects of 10 mg/kg LU-111995 in these pharmacological models of deficient PPI are most similar to those of typical antipsychotics, which are consistently effective in blocking the effects of DA agonists such as apomorphine and typically ineffective in blocking the effects of noncompetitive NMDA antagonists such as PCP (Geyer et al., 1990; Bakshi et al., 1994). It remains unclear which receptor mediates this effect. In the case of LU-111995, both receptor binding and pharmacological data indicate that DA D4 receptors are unlikely to be responsible. First, LU-111995 binds to D4 and 5-HT2A receptors with Ki values of 3.1 and 3.3 nM, respectively.
whereas the $K_i$ at the DA D$_2$ receptor is 105 nM (Steiner et al., 1998). Even at very high doses (76–100 mg/kg orally), no signs of D$_2$ receptor blockade, such as catalepsy, increased plasma prolactin levels (Steiner et al., 1998), or D$_2$ receptor-mediated increases in DA turnover were detected (data on file, Knoll AG). Two hours after oral administration of 100 mg/kg LU-111995, D$_2$ receptor occupancy was determined as 30 to 40% (data on file, Knoll AG). Unfortunately, there is no established parameter, known so far, which is linked specifically to D$_4$ receptor activation or blockade in vivo. However, it is concluded by analogy from 5-HT$_2A$ antagonism in vivo (IC$_{50}$ values of 1 and 6 mg/kg orally for inhibition of mescale-induced scratching and L-5-HTP syndrome; data on file, Knoll AG) and equal binding affinity to 5-HT$_2A$ and D$_4$ receptors in vitro (Steiner et al., 1998), that the drug blocks D$_4$ receptors after an oral dose of 10 mg/kg.

The final experiment yielded several important findings regarding isolation rearing-induced deficits in PPI and their reversal by LU-111995. First, it was found that isolation rearing of rats leads to deficits in PPI in adulthood, corroborating the findings of previous studies (Geyer et al., 1993; Wilkinson et al., 1994; Bristow et al., 1995; Varty and Higgins, 1995). Second, treatment with 10 mg/kg of LU-111995 reversed the isolation-rearing-induced deficit in PPI without having any significant effect on PPI in socially reared rats. These results confirm and extend previous reports of the reversibility of isolation rearing-induced deficits in PPI by candidate antipsychotics (Varty and Higgins, 1995; Bakshi et al., 1998; Geyer et al., 1998). Moreover, the reversals of PPI deficits achieved by LU-111995 were dissociable from increases in baseline levels of PPI, suggesting that these drugs did not normalize the PPI deficits because of a generalized improvement in PPI but rather because of a selective effect in the isolation-reared rats.

The reversal of the isolation-induced deficit in PPI by LU-111995 was found in the context of a slight, but significant decrease in startle reactivity produced by LU-111995. Nevertheless, socially reared rats treated with LU-111995 exhibited normal levels of PPI on a percentage basis, indicating that the decrease in startle magnitudes did not interfere with the demonstration of PPI.

The neuropharmacological substrates by which LU-111995 is able to reverse isolation rearing-induced deficits in PPI remain unclear. Convergent neurochemical studies implicate mesolimbic hyperdopaminergia as the putative mechanism for some behavioral effects of isolation rearing (Hall et al., 1998). Indeed, it has been shown previously that the D$_2$/D$_3$ DA antagonist, raclopride, blocks the disruption in PPI produced by isolation rearing (Geyer et al., 1993). The affinity of antipsychotics for DA receptors (Moore et al., 1993) raises the
possibility that DA receptor antagonism might mediate the ability of these drugs to reverse isolation rearing-induced deficits in PPI. Isolation rearing, however, produces changes in serotonergic and noradrenergic transmission as well (Bakshi et al., 1996; Hall et al., 1998). Hence, it is possible that effects at serotonin and norepinephrine receptors may also contribute to the ability of these compounds to normalize the isolation rearing-induced disruption in sensorimotor gating. In particular, 5-HT₂₅ antagonist M100907 also reverses the PPI deficits produced by isolation rearing (Geyer et al., 1998). Finally, it was reported recently that a selective antagonist for the glycine site on the NMDA receptor complex reversed the deficit in PPI produced by isolation rearing (Bristow et al., 1995). Thus, glutamatergic mechanisms, particularly involving NMDA receptors, may also play a role in some of the behavioral effects of isolation rearing.

The present findings indicate that isolation rearing-induced deficits in PPI are reversible by the novel compound LU-111995. LU-111995 was found to be ineffective in the PCP model, marginally effective in the amphetamine model, and clearly effective in the isolation-rearing model. The reasons for this unique profile of effects of LU-111995 remain to be determined. The isolation rearing model of deficient PPI appears to be sensitive to clinically efficacious typical and atypical antipsychotic drugs such as haloperidol, olanzapine, quetiapine, and clozapine (Geyer et al., 1993; Varty and Higgins, 1995; Bakshi et al., 1998) and might therefore provide a useful method for identifying potential novel antipsychotic drugs.

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References


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