Attenuation of Phencyclidine-Induced Object Recognition Deficits by the Combination of Atypical Antipsychotic Drugs and Pimavanserin (ACP 103), a 5-Hydroxytryptamine<sub>2A</sub> Receptor Inverse Agonist


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ABSTRACT

Subchronic administration of the N-methyl-D-aspartate receptor antagonist, phencyclidine (PCP), in rodents has been shown to produce impairment in novel object recognition (NOR), a model of visual learning and memory. We tested the hypothesis that the selective 5-HT<sub>2A</sub> inverse agonists, pimavanserin and (R)-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)-4-piperidinemethanol (M100907), would potentiate subeffective doses of atypical antipsychotic drugs (APDs) to reverse the NOR deficits. Female rats received vehicle or PCP (2 mg/kg b.i.d.) for 7 days, followed by a 7-day washout. Pimavanserin (3 mg/kg) or M100907 (1 mg/kg) alone, or four atypical APDs, risperidone (0.05–0.1 mg/kg), melperone (1–3 mg/kg), olanzapine (1–3 mg/kg) or M100907 (1 mg/kg) alone, or four atypical APDs, risperidone (0.05–0.1 mg/kg), melperone (1–3 mg/kg), olanzapine (1–2 mg/kg), or N-desmethylclozapine (1–2 mg/kg), and the typical APD, haloperidol (0.05–0.1 mg/kg), were administered alone, or in combination with pimavanserin or M100907, before NOR testing. The exploration times of objects during 3-min acquisition and retention trials, separated by a 1-min interval, were compared by analysis of variance. Vehicle-, but not PCP-treated, animals, explored the novel object significantly more than the familiar in the retention trial (p < 0.05–0.01). Pretreatment with the higher doses of the atypical APDs, but not pimavanserin, M100907, or haloperidol alone, reversed the effects of PCP. The effect of risperidone was blocked by haloperidol pretreatment. Coadministration of pimavanserin or M100907, with ineffective doses of the atypical APDs, but not haloperidol, also reversed the PCP-induced deficit in NOR. These results support the importance of 5-hydroxytryptamine<sub>2A</sub> receptor blockade relative to D<sub>2</sub> receptor blockade in the ability of atypicals to ameliorate the effect of subchronic PCP, a putative measure of cognitive dysfunction in schizophrenia.

Deficits in multiple domains of cognition, including visual learning and memory, is a characteristic feature of schizophrenia (Meltzer and McGurk, 1999). Atypical antipsychotic drugs (APDs), but less so typical APDs, have usually, but not always, been found to produce significant improvement in some domains of cognition in patients with schizophrenia (Meltzer and McGurk, 1999; Keefe et al., 2007). These classes of APDs differ in the relatively higher affinity for 5-hydroxytryptamine<sub>2A</sub> compared with dopamine (DA) D<sub>2</sub> receptors of the atypical APDs (Meltzer et al., 1989; Schotte et al., 1996; Meltzer and Huang, 2008). Microdialysis studies in rats have shown that the higher affinity for 5-HT<sub>2A</sub> compared with D<sub>2</sub> receptors of the atypical APDs contributes to their ability to enhance cortical and hippocampal DA efflux. This effect has been suggested to be important to their ability to enhance cognitive function in human and rodent models of cognitive impairment (Moghaddam and Bunney, 1990; Kuroki et al., 1999; Liégeois et al., 2002), because hydropodaminergic activity in the cortex has been postulated to be a major factor in the cognitive impairment of schizophrenia (Goldman-Rakic and Selemom, 1997).

Hypoglutamatergic function, particularly in the frontal cortex, has also been suggested to be a major factor in the cognitive impairment of schizophrenia (Coyle, 2006). Acute and subchronic administration of noncompetitive NMDA antagonists, e.g., PCP and MK-801, has been reported to produce impairments in visual and learning memory, attention.

Abbreviations: APD, atypical antipsychotic drug; DA, dopamine; serotonin (5-HT); NDMC, N-desmethylclozapine; NMDA, N-methyl-D-aspartate; NOR, novel object recognition; PCP, phencyclidine; ACP103, pimavanserin, N-(4-fluorophenylmethyl)-N′-(1-methylpiperidin-4-yl)-N″-(4-(2-methylpropoxy)phenyl)methyl) carbamid (2R,3R)-1,2-dihydroxybutanode (2:1); MK-801, 5H-dibenzo[a,d]cyclohepten-5,10-imine (dizocilpine maleate); M100907, (R)-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)-4-piperidinemethanol.
reasoning and problem solving, working memory, and social cognition (Stefani and Moghaddam, 2002; Abdul-Monim et al., 2007; Grayson et al., 2007; Rodefer et al., 2008; McLean et al., 2009), thus providing an animal model for related deficits in schizophrenia and the efficacy of atypical APDs to partially improve those deficits. Clozapine, but not the typical antipsychotic, haloperidol, has been reported to reverse cognitive deficits induced by subchronic PCP treatment in novel object recognition (NOR) and attentional set-shifting tasks (Grayson et al., 2007; Hashimoto et al., 2005; Nagai et al., 2009). Subchronic treatment with PCP has been reported to decrease basal and stress-evoked DA utilization in rat and primate prefrontal cortex (Jentsch et al., 1997, 1999), whereas acute administration of PCP enhances cortical DA (Adams and Moghaddam 1998). The latter does not seem to be related to its ability to disrupt cognitive performance (Adams and Moghaddam 1998). The ability of clozapine, but not haloperidol, to reverse the cognitive impairment due to subchronic PCP treatment in rodents has also been suggested to be due to restoration of cortical dopaminergic neurotransmission (Elsworth et al., 2008). Subthreshold doses of the typical APD, haloperidol, and various atypical APDs, in combination with any of three 5-HT2A receptor inverse agonists, e.g., M100907, eplivanserin (SR-43469-B), or pimavanserin (ACP103) enhanced cortical DA efflux (Liégeois et al., 2002; Bonacorso et al., 2002; Li et al., 2005). Pimavanserin, which has high affinity for rodent and human 5-HT2A, 5-HT2C receptors with minimal affinity and functional activity for other rat and human monoaminergic receptors (Vanover et al., 2006), was recently shown to potentiate the ability of risperidone, but not haloperidol, to treat psychosis in acutely psychotic patients with schizophrenia (Meltzer et al., manuscript in preparation). If enhanced DA efflux in the cortex or hippocampus were sufficient to reverse the effects of subchronic PCP on NOR, then the combination of a 5-HT2A inverse agonist such as pimavanserin and either haloperidol or an atypical APD would be predicted to reverse the effect of subchronic PCP to impair NOR.

This study was designed to test whether pimavanserin alone, three other atypical APDs (olanzapine, melperone, and N-desmethylclozapine (NDMC), the major metabolite of clozapine) and risperidone or haloperidol, or the combination of pimavanserin and these atypical APDs or haloperidol, are able to reverse the PCP-induced deficit in NOR. We also tested whether M100907, another 5-HT2A inverse agonist (Schmidt et al., 1992), in combination with subeffective dose of risperidone could substitute for pimavanserin in improving the PCP-induced NOR deficit. Finally, we tested the prediction that haloperidol and risperidone in combination would be ineffective to overcome the effect of PCP on NOR because of excessive D2 receptor blockade produced by pre-treatment with haloperidol.

Materials and Methods

Subjects and Housing Conditions

Eighty-eight female Long-Evans rats from two separate batches (Zivic-Miller Laboratories, Porterville, PA) weighing 220 ± 15 g were used as subjects. Rats were housed in groups of five (cages measured 38 × 59 × 24 cm) and kept under standard laboratory conditions on a 12-h light/dark cycle (lights on at 7:00 AM). Temperature and humidity conditions were 21 ± 2°C and 40 to 50%, respectively. All testing was carried out in the light phase. Food and water were available ad libitum. All experiments were conducted in accordance with the Vanderbilt animal committee regulations.

Drugs and Treatment

Forty-eight rats were randomly assigned to two treatment groups: 16 were treated with vehicle (saline, intraperitoneally) and 32 were treated with PCP (2 mg/kg i.p.) twice daily for 7 days.

PCP was dissolved in distilled water and administered in a volume of 1 ml/kg i.p. Haloperidol and pimavanserin were dissol in distilled water; olanzapine and risperidone were dissolved in a small amount of 0.1 M phosphoric acid, and the pH was adjusted to 6 to 7 with 0.1 N NaOH. NDMC and M100907 were dissolved in a small amount of 0.1 M phosphoric acid, and the pH was adjusted to 6 to 7 with 0.1 N NaOH. All doses are calculated as base equivalent weight and were administered intraperitoneally. All injections were given 30 min apart. Dose and pretreatment times for haloperidol, risperidone, and olanzapine were based on previous studies of their ability to enhance cortical DA efflux (Kuroki et al., 1999). The doses of pimavanserin, NDMC, M100907, and melperone were based on previous microdialysis experiments showing potentiation of DA and acetycholine efflux in the rat brain when administered in combination with subthreshold doses of APDs (Kuroki et al., 1999; Ichikawa et al., 2002; Li et al., 2005).

Each rat was tested four times in the NOR paradigm. To reduce carryover effects, a 7-day washout period was given between each of the test sessions. The criterion for continuing to test the rats was based on mean total exploration time in the acquisition phase ≥ 8 ± 2 s. If a rat did not explore at least that amount, they were excluded from the analysis. This happened rarely and not enough to affect the ability to use the remaining animals for analysis. All experiments consisted of 6 to 8 rats.

Animal Group 1

Experiment I: Effect of Pimavanserin to Reverse PCP-Induced Deficit in NOR. After a 7-day washout period, groups of vehicle- and PCP- treated rats were treated with pimavanserin (3 mg/kg) or acute vehicle (saline) 30 min before behavioral testing in NOR.

Experiment II: Effect of Risperidone at Subeffective and Effective Dose, and Pimavanserin Augmentation to Reverse PCP-Induced Deficit in NOR. After a 7-day washout period, PCP-treated rats were administered risperidone (0.05 mg/kg or 0.1 mg/kg), alone or pimavanserin (3 mg/kg) plus 0.05 mg/kg risperidone.

Experiment III: Effect of Haloperidol with and without Pimavanserin Augmentation to Reverse PCP-Induced Deficit in NOR. After another 7-day washout period, PCP-treated rats were administered haloperidol (0.05 or 0.1 mg/kg), alone or in combination with pimavanserin (3 mg/kg) or acute vehicle (saline, intraperitoneally).

Experiment IV: Effect of Olanzapine with and without Pimavanserin Augmentation to Reverse PCP-Induced Deficit in NOR. After another 1-week washout period after each test, the same rats were tested in the NOR paradigm after treatment with olanzapine (1 mg/kg) and pimavanserin (3 mg/kg) either alone or in combination.

Animal Group 2

A second group of 40 rats were then subjected to the same PCP or vehicle treatment regime as described above. Overall, in this within-subjects design, each rat was tested four times in the NOR test. To reduce carryover effects, a 7-day washout period was given in between each of the test sessions. Our criterion for continuing to test the rats was based on mean total exploration time in the acquisition phase ≥ 8 ± 2 s. Animals were no longer tested if total exploration time was less. Haloperidol and pimavanserin were dissolved in distilled water, and olanzapine and risperidone were dissolved in a
small amount of 0.1 M phosphoric acid, and the pH was adjusted to
6 to 7 with 0.1 N NaOH.

**Experiment V: Effect of NDMC with and without Pimavanserin Augmentation to Reverse PCP-Induced Deficit in NOR.**
After the 7-day washout period, rats were tested in the NOR paradigm after treatment with NDMC (1 mg/kg), pimavanserin (3 mg/kg), or both and acute vehicle (saline, intraperitoneally). All drugs or vehicle were given 30 min before behavioral testing.

**Experiment VI: Effect of Melperone with and without Pimavanserin Augmentation to Reverse PCP-Induced Deficit in NOR.** After a 7-day washout period, the same rats were tested in the NOR paradigm after treatment with either melperone (0.1 mg/kg), or both pimavanserin (3 mg/kg) and melperone (0.1 mg/kg) and acute vehicle (saline, intraperitoneally).

**Experiment VII: Effect of M100907 Alone and Risperidone Plus M100907 to Reverse PCP-Induced Deficit in NOR.** After another 7-day washout period, the rats were treated M100907 (0.1 mg/kg) alone and with risperidone (0.05 mg/kg).

**Experiment VIII: Effect of Haloperidol Pretreatment on Effect of Risperidone to Reverse PCP-Induced Deficit in NOR.** After another 1-week washout, PCP-treated rats were given vehicle or haloperidol (0.1 mg/kg), 30 min before risperidone (0.1 mg/kg).

**Drugs**
Pimavanserin and N-desmethylclozapine was provided by Acadia Pharmaceuticals (Torrance, CA). Clozapine was obtained from Novartis (Basel, Switzerland), risperidone was from Janssen Pharmaceuticals (Antwerp, Belgium), melperone was from Pfizer (New York, NY), and M100907 was from sanofi-aventis (Bridgewater, NJ).

**Novel Object Recognition**

**Apparatus.** The object recognition test was performed in an open field comprising a square box made of Plexiglas (52 × 52 × 31 cm) placed 37 cm above the floor on an immovable stand. The floor of the box was white with black gridlines forming nine identical squares on it. All other walls were black. A video camera connected to a video recorder and monitor was positioned above the box. The objects used for the test consisted of four heavy pyramidal structures made of metal or Perspex that could not be displaced by the animals. Care was taken to ensure that these objects were not of any natural significance to the rats.

**Object Recognition Testing.** Testing was carried out according to a previously validated method (Grayson et al., 2007). The rats were familiarized to the test environment and NOR arena before the test day. Habituation consisted of placing the subjects in the empty NOR arena for 1 h, on the day before the test day (day 1). Before behavioral testing on day 2, rats were given a further 3-min habituation.

For each experimental trial after the 3-min habituation period, the rats were given two 3-min trials (T1 and T2), separated by a 1-min intertrial interval in the home cage during which the objects were changed and the arena was cleaned. In Trial 1 (T1) or the acquisition trial, the animals were allowed to explore two identical objects (A1 and A2) for 3 min. In the second trial (T2) or the retention trial, the animals explored a familiar object (A) from T1 and a novel object (B) for 3 min. The familiar object presented during T2 was a duplicate of the object presented in T1 to avoid any olfactory trails.

**Data Collection.** Behavior in all trials was recorded on video for subsequent blind scoring for the following parameters: total exploration time of both objects in the acquisition trial (s), total exploration time of objects in the retention trial (s). Object exploration is defined by animals licking, sniffing, or touching the object with the forepaws while sniffing, but not leaning against, turning around, standing, or sitting on the object. The exploration time (s) of each object in each trial was recorded by use of two stopwatches.

**Statistical Analysis.** All data are expressed as mean ± S.E.M. (n = 6–8 per group). Exploration data were analyzed by a repeated-measures two-way ANOVA. This detected the main effect of drug treatment, main effect of the task (exploration of both objects), and the interaction between drug treatment and the two trials (acquisition and retention). Further analysis by a post hoc Bonferroni’s t test was performed if a significant effect was detected by the ANOVA, which compared the time spent exploring the novel and familiar object.

**Results**

**Experiment 1**

**Effect of Pimavanserin on PCP-Induced Deficit in Acquisition Trial T1.** An overall two-way ANOVA revealed that neither subchronic PCP nor acute pimavanserin treatment in the vehicle- or PCP-treated rats produced any significant effect on object exploration in the acquisition trial of the test (F(2,15) = 3.13, p = 0.078; Fig. 1A). Rats from all treatment groups spent equal times exploring both objects in this phase of the trial. Furthermore, a one-way ANOVA on the total time spent exploring both objects, in both trials, showed an overall significant effect of pimavanserin alone in vehicle- or PCP-treated rats to increase exploration time. Identical results were obtained with perphenazine (obtained from Sigma-Aldrich, St. Louis, MO) another typical APD, at a dose of 0.2 mg/kg (study done in a third group of animals; data not presented).

![Graph A](image1.png)

**Fig. 1.** A, the effect of acute administration of pimavanserin (3 mg/kg i.p.) after subchronic PCP treatment (2 mg/kg i.p. twice a day for 7 days followed by a 7-day drug-free period) on exploration of two identical objects in the 3-min acquisition trial T1 in a NOR test in female Long-Evans rats. Data are shown as mean ± S.E.M. of exploration time (s). n = 6 to 8 rats per group. B, the effect of acute administration pimavanserin (3 mg/kg i.p.) after subchronic PCP treatment (2 mg/kg i.p. twice a day for 7 days followed by a 7-day drug-free period) on exploration of a novel and a familiar object in the 3-min retention trial T2 in a NOR test in female Long-Evans rats. Data are expressed as mean ± S.E.M. of exploration time (s). n = 6 to 8 rats per group. ***p < 0.001 significant increase in time exploring the novel compared with familiar object, Bonferroni’s t test.
Effect of Pimavanserin Alone on PCP-Induced Deficit in the Retention Trial T2. A two-way ANOVA revealed a significant interaction effect of drug treatment and object exploration time in the retention phase of the NOR task ($F_{2,18} = 18.4, p < 0.001$; Fig. 1B). Further analysis revealed that the control group (vehicle-treated animals) and vehicle + pimavanserin group had a clear preference for the novel compared with the familiar object; i.e., spent significantly ($p < 0.001$) more time exploring the novel versus familiar object (Fig. 1B). This effect was abolished in rats treated with PCP; i.e., these rats spent a similar amount of time exploring both objects. Furthermore, treatment with pimavanserin alone at 3 mg/kg did not attenuate the behavioral deficit induced by PCP. As can be seen from Fig. 1B, pimavanserin, 3 mg/kg, alone was ineffective in reversing the PCP deficit, in that the rats again spent nearly equal time exploring the novel compared with the familiar object. Identical results were obtained with perphenazine, another typical APD, at a dose of 0.2 mg/kg (data not presented).

Experiment 2

Effect of Risperidone and Pimavanserin in Acquisition Trial T1. An overall two-way ANOVA demonstrated that neither risperidone alone nor its coadministration with pimavanserin produced a significant effect on left- and right-object exploration in the acquisition trial in vehicle- or PCP-treated rats. All treatment groups spent equivalent time exploring both identical objects in this phase of the test ($F_{4,27} = 1.54, p < 0.05$; Fig. 2A).

Effect of Risperidone and Pimavanserin in Retention Trial T2. A two-way ANOVA revealed a significant interaction between drug treatment and object exploration time in the retention phase of the NOR task ($F_{4,27} = 5.44, p < 0.001$). Risperidone alone (0.1 mg/kg) significantly improved the PCP-induced NOR deficit ($p < 0.05$), but risperidone (0.05 mg/kg) did not. However, risperidone (0.05 mg/kg) in combination with pimavanserin significantly restored greater exploration time of the novel object ($p < 0.001$; Fig. 2B).

Effect of Olanzapine and Pimavanserin in Retention Trial T2. The overall two-way ANOVA revealed a statistically significant interaction effect of drug treatment ($F_{4,32} = 5.18$; Fig. 3B). Olanzapine (1 mg/kg) when administered alone did not attenuate NOR, whereas a higher dose (2 mg/kg) significantly improved the deficit ($p < 0.05$). However, olanzapine (1 mg/kg), in combination with pimavanserin (3 mg/kg), reversed the PCP-induced deficit in exploration time of the novel relative to the familiar object ($p < 0.01$).

Experiment 3

Effect of Olanzapine and Pimavanserin in Acquisition Trial T1. An overall two-way ANOVA showed a significant interaction between object exploration and drug treatment ($F_{4,32} = 9.89, p < 0.001$; Fig. 3A). Although all treatment groups spent equal times exploring both objects in this phase of the trial, there was an overall effect of drug treatment in that coadministration of pimavanserin (3 mg/kg) with olanzapine (1 mg/kg) significantly increased the total object exploration time compared with the other groups ($p < 0.001$).

Effect of NDMC and Pimavanserin in Acquisition Trial T1. Overall ANOVA revealed no effect of drug treatment on exploration time of either object in the acquisition trial by pimavanserin or NDMC ($F_{4,30} = 2.27, p = 0.05$).

Effect of NDMC and Pimavanserin in Retention Trial T2. An overall ANOVA of the exploration times in the retention phase showed a significant interaction between drug treatment and object exploration ($F_{4,30} = 3.37, p = 0.01$; Fig. 4B). There was no significant difference between time spent exploring the novel versus familiar object in PCP-treated rats that had received NDMC alone at 1 mg/kg. However, further post hoc analysis revealed that rats that had received the combination of pimavanserin (3 mg/kg) and NDMC (1 mg/kg) successfully discriminated between the novel and familiar objects ($p < 0.01$). There was a trend for

![Fig. 2.](image_url)
NDMC alone (2 mg/kg) to restore NOR in PCP-treated animals (p < 0.07; Fig. 4B).

**Experiment 5**

**Effect of Melperone in Acquisition Trial.** An overall two-way ANOVA revealed that melperone (1–3 mg/kg), alone or in combination with pimavanserin at 3 mg/kg (Fig. 5A), did not produce any significant effect on object exploration in the acquisition trial of the NOR test (F_{4,27} = 1.63, NS). Rats from all treatment groups explored the two identical objects for equal times in this phase of the trial.

**Effect of Melperone in Retention Trial.** An overall ANOVA on the exploration times in the retention trial showed a significant interaction between drug treatment and object exploration (F_{4,27} = 4.17, p = 0.01; Fig. 5B). Melperone (3 mg/kg) alone, but not melperone (1 mg/kg), restored NOR in PCP-treated animals (p < 0.001). Further post hoc analysis revealed that, although there was no significant difference between time spent exploring the novel versus the familiar object in PCP-treated rats that had received melperone alone at 1 mg/kg, rats that had received the combination of pimavanserin and melperone (1 mg/kg) successfully discriminated between the novel and familiar objects (p < 0.01).

**Experiment 6**

**Effect of M100907 and Risperidone in Acquisition Trial.** An overall two-way ANOVA once again revealed no significant effect of drug treatment on object exploration in the acquisition trial (F_{3,31} = 0.22, NS; Fig. 6A). Rats from all treatment groups spent almost equal times exploring the two identical objects.

**Effect of M100907 and Risperidone in Retention Trial.** A two-way ANOVA showed a significant interaction between object and drug treatment (F_{3,31} = 6.66; p < 0.05). This was followed by post hoc t test that showed that the combination of M100907 (0.1 mg/kg) and risperidone (0.05 mg/kg) successfully reversed the PCP-induced deficit in NOR.
(p < 0.05; Fig. 6B). This effect was similar to that of pimavanserin on subthreshold dose of risperidone (Fig. 2, A and B) in improving NOR deficits.

**Experiment 7**

**Effect of Haloperidol and Pimavanserin in the Acquisition Trial T1.** An overall two-way ANOVA revealed that neither haloperidol (0.1 mg/kg) alone, nor haloperidol (0.05 or 0.1 mg/kg), in combination with pimavanserin (3 mg/kg), produced any significant interaction effect on object exploration in the acquisition trial of the NOR test (F_{4,31} = 0.61, NS; Fig. 7A). Rats from all treatment groups spent almost equal times exploring the two identical objects in this phase of the test.

**Effect of Haloperidol and Pimavanserin in the Retention Trial T2.** An overall two-way ANOVA revealed a significant interaction effect of drug treatment in this phase of the task (F_{4,31} = 7.28, p < 0.001; Fig. 7B). Further analysis by use of post hoc t tests revealed a significant difference between time spent exploring the novel object compared with the familiar object only in the vehicle- treated group (p < 0.01). Haloperidol 0.1 mg/kg did not affect the exploration times of the PCP-treated rats in the retention phase. Furthermore, haloperidol 0.05 or 0.1 mg/kg in combination with pimavanserin (3 mg/kg) did not affect the exploration times of the PCP-treated rats, in the retention phase.

**Experiment 8**

**Effect of Haloperidol + Risperidone in Acquisition Trial T1.** An overall two-way ANOVA demonstrated that the combination of haloperidol (0.05 mg/kg) and risperidone (0.1 mg/kg) had no significant effect on left- and right-object exploration in the acquisition trial in vehicle- or PCP-treated rats. Rats from all treatment groups spent equivalent time exploring both the objects in this phase of the trial (F_{3,21} = 0.24, NS; Fig. 8A).

**Effect of Haloperidol + Risperidone in Retention Trial T2.** A two-way ANOVA revealed no significant interaction effect of drug treatment on object exploration time in the retention phase of the NOR task (F_{3,24} = 1.69, NS; Fig. 8B). However, although the interaction was not significant, there was a significant effect on exploration of novel versus familiar objects (F_{1,24} = 9.05; p < 0.01). Post hoc analysis revealed that vehicle-treated animals spent more time ex-
Fig. 7. A, the effect of acute administration of haloperidol (0.1 mg/kg i.p.) and haloperidol (0.05–0.1 mg/kg) + pimavanserin (3 mg/kg), after subchronic PCP treatment (2 mg/kg i.p. twice a day for 7 days followed by a 7-day drug-free period) on exploration of two identical objects in the 3-min acquisition trial T1 in a NOR test in female Long-Evans rats. Data are shown as mean ± S.E.M. of exploration time (s). n = 6 to 8 rats per group. B, the effect of acute administration haloperidol (0.1 mg/kg, i.p.) and haloperidol (0.05–0.1 mg/kg) + pimavanserin (3 mg/kg), after subchronic PCP treatment (2 mg/kg i.p. twice a day for 7 days followed by a 7-day drug-free period), on exploration of a novel and a familiar object in the 3-min retention trial T2 in a NOR test in female Long-Evans rats. Data are expressed as mean ± S.E.M. of exploration time (s). n = 6 to 8 rats per group. **, p < 0.01: significant increase in time exploring the novel compared with familiar object, Bonferroni’s t test.

Fig. 8. A, the effect of acute administration of risperidone (0.1 mg/kg) and haloperidol (0.05 mg/kg) + risperidone (0.1 mg/kg), after subchronic PCP treatment (2 mg/kg i.p. twice a day for 7 days followed by a 7-day drug-free period) on exploration of two identical objects in the 3-min acquisition trial T1 in a NOR test in female Long-Evans rats. Data are shown as mean ± S.E.M. of exploration time (s). n = 6 to 8 rats per group. B, the effect of acute administration risperidone (0.1 mg/kg) and haloperidol (0.05 mg/kg) + risperidone (0.1 mg/kg), after subchronic PCP treatment (2 mg/kg i.p. twice a day for 7 days followed by a 7-day drug-free period), on exploration of a novel and a familiar object in the 3-min retention trial T2 in a NOR test in female Long-Evans rats. Data are expressed as mean ± S.E.M. of exploration time (s). n = 6 to 8 rats per group. *, p < 0.05: significant increase in time exploring the novel compared with familiar object, Bonferroni’s t test.

Exploring the novel compared with the familiar object (p < 0.05). This effect was abolished in the PCP-treated animals, as in all previous trials, and was reinstated by risperidone (p < 0.05). It is noteworthy that pretreatment with 0.05 mg/kg haloperidol blocked the ability of 0.1 mg/kg risperidone to restore the ability of PCP-treated rats to distinguish novel from familiar objects (Fig. 8B).

Discussion

The major findings of this study are that the atypical APDs, olanzapine, melperone, NDMC, and risperidone, but not the typical APD, haloperidol, significantly reversed the impairment in the retention phase of NOR subsequent to subchronic treatment with PCP in female rats. The selective 5-HT2A inverse agonists, pimavanserin and M100907, alone, at doses that fully occupy 5-HT2A receptors (Kehne et al., 1998; Vanover et al., 2006) did not attenuate the NOR deficit in PCP-treated rats. However, the combination of pimavanserin with doses of the four atypical APDs, which alone had no effect, but not the typical APD haloperidol or perphenazine (data not shown), restored NOR performance in the PCP-treated rats. M100907, another 5-HT2A inverse agonist, and a subeffective dose of risperidone were also able to reverse the effect of subchronic PCP on the retention phase of the NOR. Furthermore, it was found that haloperidol pretreatment blocked the ability of risperidone to reverse PCP-induced deficits in NOR.

The same animals were used for up to four studies, separated by a week’s time, in this, as well as previous studies (Grayson et al., 2007). There was no evidence that the subjects in the study, with or without PCP treatment, were able to remember prior exposure to the objects based on performance during the acquisition phase, which did not vary during the month. We validated this methodology by conducting experiment 8 as the last of four experiments with one group of animals and the first experiment in another group of animals, and obtained the same results (data not presented).
The doses of the atypical APDs studied here that were effective to reverse the impairment in NOR are those we had previously shown to enhance cortical DA efflux in normal rats (Kuroki et al., 1999; Ichikawa et al., 2002; Li et al., 2005). The doses of haloperidol or perphenazine are also effective to block the effects of amphetamine on locomotor activity and produce small increases in cortical DA efflux (Kuroki et al., 1999). The dose of pimavanserin used here has been reported to achieve essentially 100% 5-HT2A receptor occupancy (Vanover et al., 2006). Clozapine also reverses the effect of subchronic PCP by use of the same pretreatment schedule (Grayson et al., 2007) and produces a marked increase in cortical DA efflux (Moghaddam and Bunney, 1990; Ichikawa et al., 2001). Another atypical antipsychotic drug, lurasidone, also produces a dose-dependent reversal of the effect of subchronic PCP on NOR (Horiguchi and Meltzer, unpublished data). Quetiapine and aripiprazole, two other atypical antipsychotic drugs, also reverse the effect of a subchronic PCP regimen on NOR in rodents (Nagai et al., 2009; Tanibuchi et al., 2009). The ability of eight atypical antipsychotic drugs, which are 5-HT2A antagonists, to reverse the effects of subchronic PCP suggests that this may be a general property of atypical antipsychotic drugs that achieve 5-HT2A and D2 receptor blockade. The results reported here are consistent with the ability of clozapine, the prototypical atypical APD, to reverse the effect of subchronic treatment with PCP in rodents or monkeys in a variety of tests that require learning and memory (Hashimoto et al., 2005; Grayson et al., 2007; Elsworth et al., 2008; McLean et al., 2009). However, Rodefer et al. (2008) reported that sertindole, another serotonin-dopamine multim receptor antagonist, but not clozapine, olanzapine, or risperidone, was able to reverse the effect of subchronic PCP on an attentional set-shifting task in rats.

To further clarify the importance of the ratio of 5-HT2A to D2 receptor blockade as a component of the action of atypical APDs to reverse the PCP-induced NOR deficit, doses of the atypical APDs were decreased 50 to 67%, to reduce occupancy of both D2 and 5-HT2A receptors, at which point they were ineffective to reverse the NOR deficit. At these doses, the occupancy of rat cortical 5-HT2A receptors by risperidone and olanzapine are ~50 and ~60%, respectively, whereas those of striatal D2 receptors are ~15 and ~50%, respectively (Schotte et al., 1996). These occupancies are significantly lower than the levels thought to be necessary to treat patients with schizophrenia (Nordström et al., 1995). The ability of pimavanserin and M100907 to restore the activity of the atypical APDs suggests that more complete blockade of 5-HT2A receptors is required for them to be effective to reverse the effects of PCP. It is noteworthy that M100907 and pimavanserin effectively block the effects of acute PCP stimulation of locomotor activity, considered a model of psychosis (Maurel-Remy et al., 1995; Vanover et al., 2006; Gardell et al., 2007) as well as their ability to block NMDA antagonist effects on the firing rate of pyramidal neurons (Wang and Liang 1998). The 5-HT2A receptor has been shown to play a key role in the transport and dynamic regulation of NMDA receptors in cortical pyramidal neurons (Yuen et al., 2008). 5-HT2A agonists which are hallucinogens modulate NMDA receptor-mediated neurotransmission in the prefrontal cortex, effects that are blocked by M100907 (Arvanov et al., 1999). The effect of 5-HT2A receptor blockade to modulate glutamatergic currents may contribute to the procognitive effects of atypical APDs and selective 5-HT2A inverse agonists. The importance of 5-HT2A receptor blockade to the action of atypical APDs to counteract NMDA-receptor action in NOR is consistent with the extensive evidence linking 5-HT2A receptor mechanisms to glutamatergic function.

The ability of pimavanserin to restore the efficacy of atypical APDs after subchronic PCP treatment indicates that 5-HT2A receptor stimulation persists even after subchronic PCP treatment. Choi et al. (2009) recently reported that the PCP regimen used here did not alter levels of 5-HT2A, D2 or D4 receptors, whereas down-regulating D2 receptors in the caudate putamen and increasing 5-HT1A receptor binding in the medial prefrontal cortex and dorsolateral cortex. We have shown that 5-HT1A agonism and 5-HT2A blockade are synergistic actions of atypical APDs (Ichikawa et al., 2001). Pimavanserin and M100907 alone were ineffective in restoring performance in the NOR test in the PCP-treated rodents, indicating that reversing cognitive impairment caused by subchronic PCP treatment, unlike blocking the effect of acute PCP on neuronal firing and locomotor activity, requires more than 5-HT2A receptor blockade. The inability of low-dose haloperidol plus pimavanserin to reverse the effect of subchronic PCP treatment indicates that reversal of the impairment of NOR requires more than D2 and 5-HT2A receptor blockade, which rules out enhanced cortical DA efflux as being sufficient as well. Preliminary studies from this laboratory have found that, even after subchronic PCP, risperidone is able to enhance cortical DA efflux to an extent similar to that of control rats, but the effect of haloperidol and pimavanserin in the PCP-treated rats has not yet been studied (S. Snigdha and H. Y. Meltzer, unpublished data). However, because pretreatment with haloperidol blocked the effect of risperidone to reverse the effect of PCP treatment on NOR, without compromising motor function, it is apparent that the extent of D2 receptor blockade to that of 5-HT2A receptor blockade, is a relevant factor.

Taken together, these results indicate that more potent blockade of 5-HT2A than D2 receptor blockade is necessary, but not sufficient, for reversal of PCP-induced impairment by an atypical APD of the 5-HT2A/D2 type. It is likely that additional pharmacological features of the atypical APDs not shared by haloperidol contribute to their effectiveness as monotherapy, and their potentiation after pretreatment with pimavanserin. Some of the atypical APDs that reverse the effects of PCP have D2 and D4 receptor affinities that might possibly contribute to their efficacy even at the subthreshold dose (see Table 1 for Ki values). Wang et al. (2006) reported that 3 days of treatment with PCP, as well as acute PCP, impaired D2 receptor modulation of NMDA receptors and D4 regulation of Ca2+/calmodulin-dependent kinase II activity. The effect of PCP on D4-mediated stimulation of NMDA receptors was reversed by clozapine (Wang et al., 2006). It is possible that the atypical APDs do not use the same mechanisms to potentiate 5-HT2A and D2 receptor blockade to reverse the NOR impairment due to PCP. It is noteworthy that a variety of mechanisms, including but not limited to D1 agonism, a-adrenoceptor agonism, PDE10A inhibition, a7-nicotinic receptor agonism (Hashimoto et al., 2008), glycine transporter-1 (GlyT-1) inhibition, have been shown to reverse the effects of subchronic PCP treatment. Further study is needed to integrate these mechanisms into a unified
model. This might lead to more effective treatments for cognitive impairment in schizophrenia.

In conclusion, atypical, but not typical, APDs are able to restore the deficit in NOR memory produced by subchronic PCP treatment in rodents. This restoration requires extensive blockade of 5-HT2A receptors, which may be achieved by coadministration of 5-HT2A receptor inverse agonists, and subtherapeutic doses of atypical APDs. These findings may be relevant to the disputed ability of atypical APDs to improve declarative memory in schizophrenia. Extensive blockade of D2 receptors prevents the efficacy of atypical APDs in this model, which may have implications for combining typical and atypical drugs in patients with schizophrenia.

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References


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