The Role of 5-Hydroxytryptamine 7 Receptors in the Phencyclidine-Induced Novel Object Recognition Deficit in Rats

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ABSTRACT

The role of 5-hydroxytryptamine (serotonin) (5-HT)7 receptor antagonism in the actions of atypical antipsychotic drugs (APDs), e.g., amisulpride, clozapine, and lurasidone, if any, is uncertain. We examined the ability of 5-HT7 receptor antagonism alone and as a component of amisulpride and luraside to reverse deficits in rat novel object recognition (NOR) produced by subchronic treatment with the N-methyl-d-aspartate receptor antagonist phencyclidine (PCP), and we examined the ability of supplemental 5-HT7 antagonism to augment the inability of sulpiride, haloperidol, and (1R,4R,5S,6R)-4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY379268), a metabotropic glutamate receptor (mGlur) 2/3 agonist, which lack 5-HT7 antagonism, to reverse the NOR deficit. The 5-HT7 receptor antagonist, (2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrroldine (SB269970) (0.1–1 mg/kg) dose-dependently reversed PCP-induced NOR deficits. In addition, the ability of luraside (0.1 mg/kg) and amisulpride (3 mg/kg) to reverse this deficit was blocked by cotreatment with the 5-HT7 receptor agonist (2S)-(-)-5-(1,3,5-trimethylpyrazol-4-yl)-2-(dimethylamino)tetralin (AS19) (5–10 mg/kg), which did not affect NOR in naive rats. Sulpiride, a less potent 5-HT7 antagonist than amisulpride, did not itself improve the PCP-induced NOR deficit. However, a subeffective dose of SB269970 (0.1 mg/kg) in combination with subeffective doses of luraside (0.03 mg/kg), amisulpride (1 mg/kg), or sulpiride (20 mg/kg), also reversed the PCP-induced NOR deficit. Pimavanserin, a 5-HT2A inverse agonist, LY379268, and haloperidol did not potentiate the ability of subeffective SB269970 to improve the NOR deficit. Furthermore, the mGlur2/3 antagonist (2S)-2-amino-2-[15(2S,5S)(2-carboxycycloprop-1-yl)-3-(xanth-9-yl)propanoic acid (LY341496), which blocks the effect of clozapine to reverse the NOR deficit, did not block the SB269970-induced amelioration of the NOR deficit. These results suggest 5-HT7 antagonism may contribute to the efficacy of some atypical APDs in the treatment of cognitive impairment in schizophrenia and may itself have some benefit in this regard.

Introduction

Deficits in multiple domains of cognition, including visual learning and declarative memory, are present in most patients with schizophrenia (Saykin et al., 1991; Meltzer and McGurk, 1999). There is evidence that atypical antipsychotic drugs (APDs), which are more potent 5-hydroxytryptamine (serotonin) (5-HT)2A than dopamine (DA) D2 antagonists, are more effective than typical APDs in attenuating some of these deficits (Hagger et al., 1993; Meltzer and McGurk, 1999; Woodward et al., 2005), although not all studies are in agreement (Keefe et al., 2007). Amisulpride, another type of

ABREVIATIONS: APD, antipsychotic drug; CIs, cognitive impairment in schizophrenia; DA, dopamine; DI, discrimination index; 5-HT, 5-hydroxytryptamine (serotonin); LE, Long-Evans; mGlur, metabotropic glutamate receptor; MK-801, 5-H-dibenzo[a,d]cyclohepten-5,10-imine (dizocilpine); NMDA-R, N-methyl-d-aspartate receptor; NOR, novel object recognition; PCP, phencyclidine; ANOVA, analysis of variance; LY341495, (2S)-2-amino-2-[1S,2S]-2-carboxycycloprop-1-yl]-3-(xanth-9-yl)propanoic acid; LY379268, (1R,4R,5S,6R)-4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid; SB269970, (2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrroldine; AS19, (2S)-(-)-5-(1,3,5-trimethylpyrazol-4-yl)-2-(dimethylamino)tetralin; DR4004, 2a-[4-(4-phenyl-1,3,5,6-tetrahydropyridyld)butyl]-2a,3a,4,5-tetrahydrobenzo[c]indenol-2-(1H)-one; M109097, (R)-(-)-a-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-pipidinemethanol; SB258741, R-(-)-1-(toluene-3-sulfonfonyl)-2-[2-(4-methylpiperidin-1-yl)ethyl]pyrroldine.
Hypoglutamatergic activity has been postulated to be a major cause of cognitive impairment in schizophrenia (CIS) (Goldman-Rakic and Seleson, 1997; Coyle, 2006). Important evidence that a deficit in glutamatergic function may be the basis for this impairment in schizophrenia is that noncompetitive N-methyl-D-aspartate receptor (NMDA-R) antagonists such as phencyclidine (PCP), dizocilpine (S-Dibenzo[a,d]cyclohepten-5,10-imine; MK-801), and ketamine induce schizophrenia-like cognitive impairment in healthy subjects (Javitt and Zukin, 1991; Krystal et al., 1999). There are no definitive data on the effect of these agents on cognition in patients with schizophrenia because of ethical constraints about administering these drugs to patients with this illness. The effects of NMDA-R antagonists on cognitive function in rodents and monkeys has been intensively studied as an animal model of CIS (Gunduz-Bruce, 2009). Acute or subchronic administration of PCP and MK-801 to rodents produces cognitive impairments that model CIS [e.g., in novel object recognition (NOR); see Neill et al., 2010; Meltzer et al., 2011 for further discussion]. Atypical APDs (e.g., clozapine), but not the typical APD haloperidol, have been reported to reverse cognitive deficits induced by subchronic PCP treatment in NOR (Grayson et al., 2007; Snigdha et al., 2010). We recently reported that 5-HT2A antagonists (e.g., pimavanserin) and the mGluR2/3 agonist LY379268 (1-[2S(2S)-3-(xanth-9-yl)propanoic acid]-2-[4-methyl-1-piperidinyl]ethyl)pyrrolidine), a selective 5-HT2A antagonist (Lovell et al., 2000), has been reported to block amphetamine- and ketamine-induced locomotor activity, which is indicative of antipsychotic potential (Galici et al., 2008). 5-HT7 receptors have also been postulated to be important for learning and memory (Hedlund and Sutcliffe, 2004; Thomas and Hagan, 2004). Amisulpride, an atypical APD long thought to be a selective D2/D3 antagonist (Schoemaker et al., 1997), has been shown to be a potent 5-HT7 antagonist whose antidepressant effect may be caused by 5-HT7 receptor antagonism (Abbas et al., 2009).

There is conflicting preclinical evidence regarding the effect of 5-HT7 antagonists in rodent behavioral models of schizophrenia. Thus, SB269970 improved reference memory in a radial arm maze (Gasbarri et al., 2008) and a PCP-induced reversal learning deficit in rats (McLean et al., 2009). Consistent with these reports, several studies have demonstrated a procognitive effect of 5-HT7 antagonists (see Discussion). In contradiction to these findings is the suggestion that 5-HT7 agonism may also be an effective target for improvement of cognitive impairment (Eriksson et al., 2008), e.g., the 5-HT7 agonist AS19 [(2S)/(S)-5-(1,3,5-trimethylpyrazol-4-yl)-2-(dimethylamino)tetralin] enhanced memory formation in an autoshaping task, whereas SB269970 did not (Perez-Garcia and Meneses, 2005). Thus, there is a need for additional investigation of the role of 5-HT7 agents in CIS models.

This study had two primary aims: 1) to test the effect of the 5-HT7 receptor antagonist SB269970 on the cognitive deficit in NOR induced by subchronic PCP and 2) to elucidate the involvement of 5-HT7 receptor antagonism in the improvement in the PCP-induced NOR deficit by lurasidone, amisulpride, and sulpiride, which is of the same chemical class as amisulpride, but less potent as a 5-HT7 antagonist.

Materials and Methods

Animals. Three groups of 43 female Long-Evans (LE) rats (8 or 9 weeks old) (Harlan, Indianapolis, IN) were used as subjects for NOR experiments. The first 43 rats (rat group 1) were used for experiments 1 and 2; the second 43 rats (rat group 2) were used for experiments 3 to 5; and the third 43 rats (rat group 3) were used for experiments 6 to 8. LE rats were housed in groups of three or four on a 12-h light/dark cycle. Food and water were available ad libitum. All experiments were conducted during the light phase in accordance with Vanderbilt animal committee regulations.

Drugs. Lurasidone was provided by Dainippon Sumitomo Pharma (Osaka, Japan). Pimavanserin was provided by Acadia Pharmaceuticals (Torrence, CA). Haloperidol and sulpiride were obtained from Sigma-Aldrich (St Louis, MO). Amisulpride, AS19, LY379268, LY341495 [(2S)-2-amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl)propanoic acid], and SB269970 were purchased from Tocris Bioscience (Ellisville, MO). PCP was supplied as a generous gift from the National Institute of Drug Abuse (Bethesda, MD). PCP, haloperidol, and pimavanserin were dissolved in distilled water. AS19 and lurasidone were dissolved in 0.5% methylcellulose and 0.2% Tween80. LY379268 and SB269970 were dissolved in saline. LY341495 was dissolved in a small amount of 0.1 M sodium hydroxide and then diluted with saline. Amisulpride and sulpiride were dissolved in a small amount of 0.1 M phosphoric acid, and the pH was adjusted toward neutral (pH 6–7) with 0.1 N NaOH. All drugs or vehicle were administered intraperitoneally in a volume of 1 mg/kg body weight 30 min before NOR testing.

Drug Treatment. LE rats (rat groups 1 and 3) were randomly assigned to two treatment groups: nine were treated with vehicle (saline, intraperitoneally) and the remainder were treated with PCP (2 mg/kg i.p.) twice daily for 7 days. Rats in group 2 were randomly assigned to two treatment groups: 17 were treated with vehicle (saline, intraperitoneally), and the remainder were treated with PCP (2 mg/kg i.p.) twice a day for 7 days. Subsequently, animals were given a 7-day washout period before NOR testing.

Each rat was tested two or three times as in previous studies (Grayson et al., 2007; Snigdha et al., 2010). To reduce carryover effects, a 7-day washout period was given between each of the test
sessions. If the exploration time in the acquisition or retention trials to either of two objects was less than 5 s, the data were excluded from analysis. This rarely occurred and did not affect the ability to complete the analysis using the data from the remaining animals of that group. All experimental groups consisted of six to nine rats.

**NOR Test.** Testing was carried out according to a previously validated method (Snigdha et al., 2010). In brief, all rats were habituated for 1 h to the NOR arena for 3 consecutive days before the first NOR test. Rats were given an additional 3-min habituation on the day of testing. After the 3-min habituation, the rats were given two 3-min trials (an acquisition trial and a retention trial) separated by a 1-min intertrial return to their home cage. During the acquisition trial, the animals were allowed to explore two identical objects (A1 and A2). During the retention trial, the animals explored a familiar object (A) from the acquisition trial and a novel object (B).

Behavior was recorded on video for blind scoring of object exploration. Object exploration was defined as an animal licking, sniffing, or touching the object with the forepaws while sniffing. The exploration time (s) of each object in each trial was recorded manually by the use of two stopwatches. The discrimination index (DI) \((time spent exploring the novel object - time spent exploring the familiar object)/total exploration time\) was then calculated for retention trials.

**Data Analysis.** All data are expressed as the mean ± S.E.M. \((n = 6–9 per group)\). Exploration data were analyzed by a repeated-measures two-way analysis of variance (ANOVA) followed by the pair-wise comparison when a significant effect was detected by the ANOVA. DI data were analyzed by one-way ANOVA followed by the Bonferroni test when a significant effect was detected by the ANOVA.

**Results**

**Effect of SB269970 in Subchronic PCP-Treated Rats (Experiment 1).** In the acquisition trial, no significant differences in time spent exploring the two identical objects were observed in any group (Fig. 1A). There were no significant effects of drugs during the acquisition trial period in any of the experiments (Supplemental Figs. 1–7). In the retention phase, vehicle-treated animals explored the novel object significantly longer than the familiar object \((p < 0.001; \text{Fig. 1B})\). The ability to discriminate novel and familiar objects was abolished by subchronic PCP treatment. SB269970 (0.1 and 0.3 mg/kg) failed to reverse this deficit (Fig. 1B), although exploration time with novel objects in SB269970 (0.3 mg/kg)-treated rats was numerically longer than that with familiar objects \((9.7 ± 2.7 and 5.1 ± 0.7 s, respectively)\). However, the highest tested dose of SB269970 (1 mg/kg) significantly attenuated the NOR deficit \((p < 0.05; \text{Fig. 1B})\). The DI was significantly reduced after subchronic PCP treatment \((p < 0.01)\), and SB269970 at 1 mg/kg, but not at 0.1 and 0.3 mg/kg, significantly \((p < 0.05)\) reversed the reduction in DI (Fig. 1C). SB269970 alone significantly decreased the total exploration time (sum of acquisition time plus retention time) in the subchronic PCP-treated rats \((p < 0.001)\).

**Effect of AS19 Plus Lurasidone on Subchronic PCP-Treated Rats (Experiment 2).** In the retention trial, vehicle-treated animals showed preference for the novel object \((p < 0.05; \text{Fig. 2A})\). PCP-treated rats did not show preference for the novel object (Fig. 2A). Lurasidone (0.1 mg/kg) significantly attenuated the PCP-induced deficit \((p < 0.001; \text{Fig. 2A})\). Rats treated with lurasidone (0.1 mg/kg) and AS19 (5 or 10 mg/kg) did not show novel object preference (Fig. 2A). Statistical analysis showed subchronic PCP treatment significantly reduced the DI \((p < 0.05; \text{Fig. 2B})\). Lurasidone alone (0.1 mg/kg), but not lurasidone (0.1 mg/kg) plus AS19 (5 mg/kg), reversed the PCP-induced deficit \((p < 0.001; \text{Fig. 2B})\).

**Fig. 1.** Effect of acute administration of SB269970 (SB; 0.1, 0.3, and 1 mg/kg) on PCP-induced cognitive impairment in the NOR test. A, effect of SB269970 (0.1, 0.3, and 1 mg/kg i.p.) on exploration of two identical objects in the acquisition trial in the NOR test. Data are shown as mean ± S.E.M. \((n = 7–9 per group)\). No significant effect of drugs was seen in the acquisition trials in any of the experiments (Supplemental Figs. 1–7). B, effect of SB269970 (0.1, 0.3, and 1 mg/kg i.p.) on exploration of a novel and a familiar object in the retention trial in the NOR test. Data are shown as mean ± S.E.M. \((n = 7–9 per group)\). No significant effect of drugs was seen in the retention trials in any of the experiments (Supplemental Figs. 1–7). C, Effect of SB269970 (0.1, 0.3, and 1 mg/kg i.p.) on the DI. Data are shown as mean ± S.E.M. \((n = 7–9 per group)\). **+, p < 0.001; **, p < 0.05, significant difference in time spent exploring the novel compared with the familiar object. #, p < 0.01, significant decrease in DI compared with the vehicle. #, p < 0.05, significant reversal in DI compared with the PCP group.
Fig. 2. Effect of acute administration of lurasidone (LUR; 0.1 mg/kg) with or without AS19 (5 and 10 mg/kg) on PCP-induced cognitive impairment in the NOR test. A, effect of lurasidone (0.1 mg/kg i.p.) and lurasidone (0.1 mg/kg i.p.) plus AS19 (5 or 10 mg/kg i.p.) on exploration of a novel and a familiar object. B, effect of lurasidone (0.1 mg/kg) with or without AS19 (5 and 10 mg/kg) on the DI. Data are shown as mean ± S.E.M (n = 6–8 per group). ***p < 0.001; *p < 0.05, significant difference in time spent exploring the novel compared with the familiar object. B, effect of lurasidone (0.1 mg/kg) with or without AS19 (5 and 10 mg/kg) on the DI. Data are shown as mean ± S.E.M (n = 6–8 per group). *p < 0.05, significant decrease in DI compared with the vehicle. #p < 0.05, significant reversal in DI compared with the lurasidone (0.1 mg/kg) group.

or 10 mg/kg), improved the reduction of DI (p < 0.05; Fig. 2B). AS19 (10 mg/kg) significantly attenuated the effect of lurasidone to reverse the PCP-induced DI reduction (p < 0.05; Fig. 2B).

Effect of SB269970 Plus Lurasidone on Subchronic PCP-Treated Rats and Effect of AS19 on Control Rats (Experiment 3). In the retention trial, vehicle-treated rats showed exploratory preference for the novel object, and AS19 (10 mg/kg) did not affect the preference (p < 0.05 and 0.001, respectively; Fig. 3A). In PCP-treated rats, there was no significant difference between the time spent exploring the novel and the familiar objects (Fig. 3A). Lurasidone (0.03 mg/kg) plus 0.1 mg/kg SB269970, but not 0.03 mg/kg lurasidone alone, significantly reversed the PCP-induced NOR deficit (p < 0.01; Fig. 3A). The DI was significantly reduced after subchronic PCP treatment (p < 0.001); treatment with 0.03 mg/kg lurasidone plus 0.1 mg/kg SB269970, but not 0.03 mg/kg lurasidone alone, significantly improved the DI reduction (p < 0.01; Fig. 3B).

Effect of SB269970 Plus LY379268 or LY341495 on Subchronic PCP-Treated Rats (Experiment 4). In the retention trial, vehicle-treated rats showed preference for the novel object (p < 0.001), and this preference was abolished in PCP-treated rats (Fig. 4A). SB269970 (0.1 mg/kg) plus 1 mg/kg LY379268 did not affect the exploration times of the PCP-treated rats (Fig. 4A). SB269970 (1 mg/kg) and 1 mg/kg LY341495 successfully reversed the PCP-induced deficit (p < 0.001; Fig. 4A). Subchronic PCP treatment significantly reduced the DI compared with control rats (p < 0.001; Fig. 4B).
SB269970 (0.1 mg/kg) plus 1 mg/kg LY379268 did not affect the PCP-induced reduction in DI (Fig. 4B). SB269970 (1 mg/kg) in combination with 1 mg/kg LY341495 significantly improved the PCP-induced reduction in DI (p < 0.001; Fig. 4B). The combination of SB269970 plus LY379268 significantly enhanced the total exploration time compared with the sum observed in rats treated with subchronic PCP and subsequently administered vehicle and either SB269970 or LY279268 alone (p < 0.01).

Effect of SB269970 Plus Pimavanserin or Haloperidol on Subchronic PCP-Treated Rats (Experiment 5). In the retention trial, vehicle-treated rats had a clear preference for the novel object (p < 0.001). This effect was abolished in rats treated with PCP (Fig. 5A). SB269970 (0.1 mg/kg) plus 3 mg/kg pimavanserin or 0.03 mg/kg haloperidol did not improve the PCP-induced deficit (Fig. 5A). The DI was significantly reduced after PCP treatment (p < 0.05; Fig. 5B). SB269970 (0.1 mg/kg) plus 3 mg/kg pimavanserin or 0.03 mg/kg haloperidol did not reverse the reduction of the DI in PCP-treated rats (Fig. 5B). The combination of SB269970 and either pimavanserin or haloperidol had no effect on total exploration time.

Effect of Amisulpride and AS19 in Subchronic PCP-Treated Rats (Experiment 6). In the retention phase, vehicle-treated animals explored the novel object significantly longer than the familiar object (p < 0.01; Fig. 6A). The ability to discriminate novel and familiar objects was abolished by subchronic PCP treatment, and 3 mg/kg amisulpride, but not 1 mg/kg, significantly attenuated the NOR deficit (p < 0.01;
Fig. 6A). Rats treated with 3 mg/kg amisulpride and 10 mg/kg AS19 did not show preference for the novel object (Fig. 6A). The DI was significantly reduced after subchronic PCP treatment compared with vehicle-treated animals ($p < 0.01$; Fig. 6B). Amisulpride at 3 mg/kg, but not at 1 mg/kg, significantly reversed the reduction in DI ($p < 0.01$; Fig. 6B). AS19 (10 mg/kg) significantly blocked the ability of 3 mg/kg amisulpride to reverse the PCP-induced DI reduction ($p < 0.05$; Fig. 6B).

**Effect of Subeffective Amisulpride Plus Subeffective SB269970 or Lurasidone on Subchronic PCP-Treated Rats (Experiment 7).** In the retention trials, vehicle-treated animals showed preference for the novel object ($p < 0.01$; Fig. 7A). PCP-treated rats did not show preference for the novel object (Fig. 7A). Amisulpride (1 mg/kg) did not attenuate the PCP-induced NOR deficit (Fig. 7A). Treatment with 1 mg/kg amisulpride in combination with 0.1 mg/kg SB269970 or 0.03 mg/kg lurasidone significantly improved the DI ($p < 0.001$; Fig. 7B). Amisulpride (1 mg/kg) in combination with 0.1 mg/kg amisulpride (1 mg/kg i.p.) plus SB269970 (0.1 mg/kg i.p.) or lurasidone (0.03 mg/kg i.p.) on exploration of a novel and a familiar object in the retention trial in the NOR test. Data are shown as mean ± S.E.M (n = 7–9 per group). ***, $p < 0.01$; ###, $p < 0.001$, significant decrease in DI compared with the familiar object. B, effect of amisulpride (1 mg/kg i.p.) and amisulpride (1 mg/kg i.p.) plus amisulpride (3 mg/kg i.p.) plus AS19 (10 mg/kg i.p.) on the DI. Data are shown as mean ± S.E.M (n = 7–9 per group). ***, $p < 0.01$, significant decrease in DI compared with the vehicle. ###, $p < 0.001$, significant reversal in DI compared with the amisulpride (3 mg/kg) group.

Effect of Sulpiride Plus/Minus Subeffective SB269970 on Subchronic PCP-Treated Rats (Experiment 8).** In the retention trial, vehicle-treated rats showed exploratory preference for the novel object ($p < 0.001$; Fig. 8A). In PCP-treated rats, there was no significant difference between the time spent exploring the novel and the familiar object, and sulpiride (20 and 30 mg/kg) did not attenuate this deficit (Fig. 8A). Rats treated with 20 mg/kg
significant decrease in DI compared with the vehicle.

exploration time.

combination of SB269970 and sulpiride had no effect on total p


There was a trend for 20 mg/kg sulpiride plus 1 mg/kg SB269970 mg/kg) did not reverse the DI reduction induced by PCP (Fig. 8B).

Data are shown as mean i.p.) and sulpiride (20 mg/kg i.p.) plus SB269970 (0.1 mg/kg i.p.) on the DI. The selective 5-HT7 antagonist SB269970 dose-dependently (Winters et al., 2010) and a frequently studied model of CIS. Apotential limitation of this study is that the same detecting compounds with therapeutic potential in treating CIS. It is noteworthy that subchronic treatment with the NMDA-R antagonist MK-801 nonsignificantly lowered 5-HT7 mRNA expression in rat hippocampus (Healy and Meadow-Woodruff, 1999).

We have recently reported that 5-HT2A inverse agonists [e.g., pimavanserin and (R)-(+)a-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-pipidinemethanol (M100907)] or the mGluR2/3 agonist LY379268 can also restore the ability of a subeffective dose of atypical APDs to attenuate the PCP-induced NOR deficit (Snigdha et al., 2010; Horiguchi et al., 2011). Unlike SB269970, neither agent alone, or in combination, can attenuate the effect of subchronic PCP. This suggests 5HT, antagonism is more central to the reversal of the effects of subchronic PCP in this model than either of the other two effects but that restoration of the activity of a subeffective dose of an atypical APD can be independently achieved by any one of these three mechanisms. However, a subeffective dose of SB269970, in combination with either pimavanserin or LY379268, did not improve the NOR deficit induced by PCP. In addition, the mGluR2/3 antagonist LY341495 did not block the effect of SB269970 to improve the PCP-induced NOR deficit, whereas it did block the ability of clozapine to reverse the effects of PCP on NOR (Horiguchi et al., 2011). This indicates that the combination of either 5-HT7 antagonism with 5-HT2A inverse agonism or mGluR2/3 agonism is insufficient to reverse the effects of subchronic PCP on NOR. Combined treatment with haloperidol to add D2 receptor antagonism to any of these mechanisms also does not reverse the subchronic PCP-induced NOR deficit (M. Horiguchi and H. Y. Meltzer, unpublished work). It is noteworthy that a subeffective dose of SB269970 in combination with haloperidol did not improve the PCP-induced NOR deficit. These data suggest that the effect of the atypical APDs, lurasidone and amisulpride, on the PCP-induced NOR deficit is different from that of the typical APD haloperidol, and that D2 antagonism is insufficient to potentiate the effect of SB269970 on the PCP-induced NOR deficit. However, atypical APDs that lack 5-HT7 antagonism, e.g., melperone (Roth et al., 2004) are also able to reverse the effects of subchronic PCP on NOR (Snigdha et al., 2010).

Our results confirm that subchronic PCP treatment (2 mg/kg i.p., twice a day for 7 days) induces a robust, persistent impairment in NOR (Grayson et al., 2007; Snigdha et al., 2010; Horiguchi et al., 2011). Our results provide additional support for the conclusion that this model is valuable for detecting compounds with therapeutic potential in treating CIS. A potential limitation of this study is that the same animals were used for up to three studies, separated by a 7-day washout period, raising the possibility of a carryover

sulpiride plus 0.1 mg/kg SB269970 showed preference for the novel object (p < 0.05; Fig. 8A). The DI was significantly reduced after subchronic PCP treatment (p < 0.01); sulpiride (20 and 30 mg/kg) did not reverse the DI reduction induced by PCP (Fig. 8B). There was a trend for 20 mg/kg sulpiride plus 1 mg/kg SB269970 to restore the DI in PCP-treated animals (p = 0.06; Fig. 8B). The combination of SB269970 and sulpiride had no effect on total exploration time.

Discussion

NOR is a possible analog of declarative memory in humans (Winters et al., 2010) and a frequently studied model of CIS. The selective 5-HT7 antagonist SB269970 dose-dependently reversed the PCP-induced deficit in NOR, indicating that robust 5-HT7 receptor blockade is sufficient to restore NOR

in subchronic PCP-treated rats. The 5-HT7 agonist AS19 blocked the ameliorating effect of lurasidone and amisulpride, atypical APDs with potent 5-HT7 antagonist properties, on the PCP-induced NOR deficit, whereas AS19 did not affect NOR in vehicle-treated rats. Sulpiride, a less potent 5-HT7 antagonist, did not improve PCP-induced NOR deficit by itself. Moreover, the combination of subeffective doses of SB269970 and lurasidone, amisulpride, or sulpiride, also improved the PCP-induced NOR deficit, indicating that extensive blockade of 5-HT7 receptors, most likely in the hippocampus and parahippocampus (Hedlund and Sutcliffe, 2004; Winters et al., 2008), is capable of overcoming the effect of subchronic PCP, at least on this specific cognitive function. It is noteworthy that subchronic treatment with the NMDA-R antagonist MK-801 nonsignificantly lowered 5-HT7 mRNA expression in rat hippocampus (Healy and Meadow-Woodruff, 1999).

Fig. 8. Effect of acute administration of sulpiride (SUL; 20 and 30 mg/kg) and sulpiride (20 mg/kg i.p.) plus SB269970 (SB; 0.1 mg/kg i.p.) on PCP-induced cognitive impairment in the NOR test. A, effect of sulpiride (20 and 30 mg/kg i.p.) and sulpiride (20 mg/kg i.p.) plus SB269970 (0.1 mg/kg i.p.) on exploration of a novel and a familiar object in the retention trial in the NOR test. Data are shown as mean ± S.E.M (n = 7–8 per group). ***, p < 0.001; *, p < 0.05, significant difference in time spent exploring the novel compared with the familiar object. B, effect of sulpiride (20 and 30 mg/kg i.p.) and sulpiride (20 mg/kg i.p.) plus SB269970 (0.1 mg/kg i.p.) on the DI. Data are shown as mean ± S.E.M (n = 7–8 per group). **, p < 0.01, significant decrease in DI compared with the vehicle.
effect. The 7-day interval should be sufficient to wash out any residual drug. This design, which has been used in previous studies, requires the use of fewer animals for research purposes and is, thus, both more humane and cost-effective; it has previously been shown not to affect the results (Snigdha et al., 2010). Although total exploration time is reduced during the testing at weeks 4 to 6 after initiating subchronic PCP treatment, the effect of treatments on the DI is not affected (M. Horiguchi and H. Y. Meltzer, unpublished data).

This is the first report that the selective 5-HT7 antagonist SB269970 reverses the NOR deficit induced by subchronic PCP. Our results are in accord with studies showing a procognitive effect of 5-HT7 antagonists. Gasbarri et al. (2008) reported that SB269970 improved reference memory, but not working memory, in rat radial arm maze. SB269970 at 3 and 10 mg/kg, but not at 1 mg/kg, improved PCP-induced reversal learning deficits in rats (McLean et al., 2009). The present study showed that SB269970 is more potent in reversing PCP-induced NOR impairments than in reversal learning. SB269970 and DR4004 [2a-4-(4-phenyl-1,2,3,6-tetrahydropyridyl)butyl]-2a,3,4,5-tetrahydrobenzo(c,d)indol-2-(1H)-one], another 5-HT7 antagonist, reversed amnesia induced by scopolamine and MK-801 in an autoshaping task in rats (Meneses 2004). Pouzet et al. (2002) have shown that SB258741 [R+(+-)(1-toluene-3-sulfonyl)-2-[2-(4-methylpiperidin-1-yl)ethyl]-pyrrolidine], an analog of SB269970, normalized the disruptive effect of PCP on prepulse inhibition in rats. On the other hand, other researchers found no effect of 5-HT7 antagonists in reversing various deficits induced by NMDA-R antagonists, including baseline and PCP-disrupted prepulse inhibition (Pouzet et al., 2002; Galici et al., 2008; Semenova et al., 2008). 5-HT7 receptors are located postsynaptically on GABAergic, glutamatergic, and cholinergic neurons in the cortex, hippocampus, and striatum (Vysokanov et al., 1998; Bonsi et al., 2007), and, thus, are in a position to affect cognition. Yadav et al. (2011) have recently provided evidence that the antipsychotic-like activity of clozapine and olanzapine depends on an intact presynaptic serotonergic neuronal system. Taken together, these results suggest that 5-HT7 antagonists, acting postsynaptically, attenuate some, but not all, domains of cognitive impairments as is the case with atypical APDs in patients with schizophrenia (Woodward et al., 2005). Our results add additional evidence for the hypothesis that 5-HT7 antagonism is of value in improving some aspects of CIs, especially declarative memory, secondary to NMDA-R-induced hypoglutamatergic function. Amisulpride has been shown to have a limited ability to improve some domains of cognition in patients with schizophrenia, including declarative memory (Wagner et al., 2005; Mortimer et al., 2007; Ahn et al., 2009). However, the clinical benefit in groups of patients is nowhere near as robust as the efficacy in this model. A positive association for two polymorphisms of the 5-HT7 receptors with schizophrenia has been reported in a cohort of Japanese patients with schizophrenia (Ikeda et al., 2006). The benefits of amisulpride on cognition might be more apparent when genetic factors are taken into account.

Lurasidone is a D2, 5-HT2A, 5-HT7 receptor antagonist with 5-HT1A partial agonist properties (Ishibashi et al., 2010) that has been reported to have cognitive benefits in some animal models (Ishiyama et al., 2007; Enomoto et al., 2008). We recently reported that more potent blockade of 5-HT2A than D2 receptors is necessary, but not sufficient for reversal of PCP-induced NOR impairment by atypical APDs (Snigdha et al., 2010). In this study, we found that the 5-HT7 agonist AS19 blocked the effect of lurasidone to ameliorate the PCP-induced NOR deficit. Furthermore, the combination of subeffective doses of SB269970 and lurasidone ameliorated the NOR impairment induced by PCP.

Because amisulpride lacks 5-HT2A affinity and has long been considered to be a D2/D3-selective antagonist (Schoemaker et al., 1997), the basis for its atypical antipsychotic properties are clearly unlike those of clozapine and other multireceptor antagonists. Amisulpride’s antidepressant action in animal models requires functional 5-HT7 receptors (Abbas et al., 2009). The same seems to be true for its cognitive benefits. The results with sulpiride, which has a much lower affinity for 5-HT7 receptors than amisulpride, supports this conclusion (Roth et al., 1994). Sulpiride was effective in this model only when augmented by SB269970. This contrasts with the failure of coadministration of haloperidol and SB269970 to restore the DI in subchronic PCP-treated rats, indicating important differences between sulpiride, amisulpride, and haloperidol. The differences between amisulpride and sulpiride are also apparent with regard to cortical DA efflux because only amisulpride, like clozaine, enhances cortical DA efflux (Kuroki et al., 1999). We have found that SB269970 augments the ability of a subeffective dose of lurasidone to enhance cortical and hippocampal DA efflux in awake, freely moving rats (M. Huang and H.Y. Meltzer, manuscript in preparation). Taken together, these results strongly suggest that 5-HT7 antagonism contributes to the ability of lurasidone and amisulpride to reverse subchronic PCP-induced NOR impairment and add to the evidence that 5-HT7 antagonism is a critical component to the efficacy of amisulpride (Abbas et al., 2009). Furthermore, the suggestion that the ability of amisulpride to improve cognition in patients with schizophrenia is independent of 5-HT7 receptor-mediated actions (Wagner et al., 2005) is unlikely to be correct.

AS19 did not affect the novel object preference of vehicle-treated rats. However, the NOR test in the present study used only a 1-min interval between acquisition and testing. Further study with longer intervals between the two procedures after AS19 treatment is needed. However, these results with AS19 are inconsistent with the data of Perez-Garcia and Meneses (2005), showing that AS19 reversed memory deficits induced by acute administration of MK-801 or scopolamine, a muscarinic antagonist, and improved memory consolidation in naïve rats in an autoshaping task. These discrepancies may be related to differences between normal rats and rats treated with various NMDA-R and muscarinic antagonists.

The combination of a subeffective dose of SB269970 and LY379268, but no other combination of SB269970 with a putative antipsychotic, including pimavanserin, increased exploration time. The enhanced exploration was noted for both the acquisition and retention phases. The significance of total exploration time remains to be determined. It is intriguing to consider it may be a reflection of increased interest in the environment, and thus, possibly suggestive of efficacy for some types of negative symptoms, including anergia and avolition. Because the combination did not lead to improvement in the DI, it is unlikely to be related to changes in memory function.
In conclusion, these results suggest that 5-HT, antagonism has a procognitive effect on the subchronic PCP-induced impairment in NOR and that 5-HT antagonism contributes to the ability of lurasidone and amisulpride to ameliorate the PCP-induced NOR deficit. We also conclude that sulpiride differs from amisulpride because of weaker 5-HT antagonism. Furthermore, these results suggest that 5-HT(7) antagonism, D(2) antagonism, and mGluR2/3 agonism are not necessary for 5-HT(7) receptor blockade to ameliorate the PCP-induced deficit in NOR. Thus, 5-HT(7) antagonism may be a novel approach for the improvement of cognitive impairment in some patients with schizophrenia, if indeed there is a hypoglutamatergic deficit in schizophrenia.

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Authorship Contributions
Participated in research design: Horiguchi, Huang, and Meltzer.
Conducted experiments: Horiguchi.
Contributed new reagents or analytic tools: Horiguchi and Huang.
Performed data analysis: Horiguchi.
Wrote or contributed to the writing of the manuscript: Horiguchi and Meltzer.

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