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Reversal of Phencyclidine-Induced Dopaminergic Dysregulation by *N*-Methyl-D-Aspartate Receptor/Glycine-site Agonists

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N-methyl-D-aspartate (NMDA) receptors may play a critical role in the pathophysiology of schizophrenia. In rodents, NMDA receptor antagonists, such as phencyclidine (PCP), induce dopaminergic dysregulation that resembles the pattern observed in schizophrenia. The present study investigates the degree to which concurrent treatment with NMDA modulators, such as glycine and the recently developed glycine transport antagonist *N*[3-(4″-fluorophenyl)-3-(4″-phenylphenoxy)propyl]sarcosine (NFPS) prevents dopaminergic dysregulation observed following chronic (3 months) or subchronic (2 weeks) PCP administration. Both chronic and subchronic treatment with PCP in the absence of glycine or NFPS led to significant potentiation of amphetamine-induced dopamine release in the prefrontal cortex and striatum, similar to that observed in schizophrenia. Treatment with either high-dose glycine or NFPS along with PCP prevented PCP effects. These findings demonstrate effective doses of glycine for use in animal models of schizophrenia, and support recent clinical studies showing the effectiveness of NMDA agonists in the treatment of persistent symptoms of schizophrenia.
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INTRODUCTION

Symptoms of schizophrenia have traditionally been attributed to the hyperactivity of brain dopaminergic systems (eg Davis *et al*, 1991; Moore *et al*, 1999). Over the past decade, however, the phencyclidine (PCP) model of schizophrenia has attained increasing prominence (Coyle, 1996; Javitt *et al*, 1987; Javitt and Zukin, 1990, 1991; Jentsch *et al*, 1999; Krystal *et al*, 1994; Newcomer *et al*, 1999). This model is based upon the observation that PCP and related agents such as ketamine induce schizophrenia-like symptoms by blocking *N*-methyl-D-aspartate (NMDA)-type glutamate receptors, and exacerbate symptoms in remitted patients. Further, agents that potentiate NMDA receptor-mediated neurotransmission significantly reduce persistent negative and cognitive symptoms of schizophrenia (Goff *et al*, 1999; Heresco-Levy *et al*, 1999; Javitt *et al*, 2001, 1994; Shoham *et al*, 2001; Tsai *et al*, 1998). Psychotomimetic effects of PCP are observed during both acute and chronic administration. Further, in early studies, behavioral effects of PCP were found to be most severe in patients with postencephalitic Parkinson's disease, suggesting critical interactions with brain dopaminergic systems (Meyer *et al*, 1959).

A recent finding in schizophrenia research has been the demonstration that patients show increased dopaminergic sensitivity to amphetamine challenge. This finding has been replicated in several cohorts using *in vivo* PET and SPECT imaging (Abi-Dargham *et al*, 1998; Breier *et al*, 1998, 1997; Kegeles *et al*, 1999; Laruelle *et al*, 1998, 1999, 1996, 1995, 1997a, b). Similar abnormalities are observed in humans following ketamine administration (Kegeles *et al*, 2000), and in rodents following acute (Balla *et al*, 2003; Miller and Abercrombie, 1996) or chronic (Balla *et al*, 2001b, 2003) NMDA antagonist administration.

NMDA receptors are modulated by amino acids, including glycine and D-serine, which bind to the glycine modulatory site of the NMDA complex. Both glycine (Javitt *et al*, 1999; Toth and Lajtha, 1986) and D-serine (Contreras, 1990; Nilsson *et al*, 1997) have been shown to reverse the behavioral effects of PCP in rodents following acute administration, indicating the *in vivo* relevance of the interaction. The effects of these agents during long-term administration, however, have not been evaluated. The use of D-serine in rodents is contraindicated because of nephrotoxicity (Carone and Ganote, 1975). In contrast, glycine is well tolerated in rodents for up to 5 months at

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doses that produce serum levels similar to those observed during clinical trials in schizophrenia (Shoham *et al*, 2001). The present study, therefore, evaluates the effects of long-term glycine treatment on neurochemical alterations induced by chronic PCP administration.

Occupancy of the glycine site is governed by a glycine (GlyT1) transporter that maintains low, subsaturating glycine levels in the immediate vicinity of NMDA receptors. Recently developed glycine transport inhibitors, such as glycyldodecylamide (GDA) (Javitt *et al*, 1999; Javitt and Frusciante, 1997) or *N*[3-(4″-fluorophenyl)-3-(4″-phenylphenoxy)propyl]sarcosine (NFPS) (Atkinson *et al*, 2001; Herdon *et al*, 2001), increase brain glycine levels *in vivo* (Atkinson *et al*, 2001) and potentiate NMDA receptor-mediated neurotransmission *in vitro* (Bergeron *et al*, 1998). As yet, however, few animal models have been published that show sensitivity to effects of these agents. The present study evaluates the degree to which PCP-induced enhancement of amphetamine-induced DA release in brain may be used to detect the effects of indirect, as well as direct, agonists of the NMDA-associated glycine-binding site.

MATERIALS AND METHODS

Animals

Studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. Male Sprague–Dawley rats (150–200 g) were bred in-house. Animals were maintained under a 10/14 h dark/light cycle, and were allowed food and water *ad libitum* during the microdialysis procedure and during the night-time locomotor activity measurements. Food was withdrawn during amphetamine challenge procedures. Three to nine animals were used per group.

PCP Administration

PCP hydrochloride (obtained from the National Institute of Drug Abuse) was dissolved in sterile physiological saline and administered via an osmotic pump (ALZA Corporation, model 2ML4) implanted under the skin. For 3-month administration, minipumps were replaced monthly to maintain appropriate serum levels. Saline-filled pumps were used in control animals. The pumps were filled based on the animal weight at the start of the experiment to deliver indicated PCP doses. The implantation was carried out under anesthesia with ketamine hydrochloride and acepromazine maleate 1 : 1 mixture (1 μl/g i.m.).

Microdialysis

Microdialysis was performed following either 14 days or 3 months of PCP administration, as indicated. Animals were still receiving PCP at the time of microdialysis. Animals were anesthetized with chloral hydrate (400 mg/kg i.p.) and mounted in a stereotaxic frame (David Kopf Instrument). A CMA 10 guide cannula (Carnegie Medicine) was implanted into the left dorsomedial striatum and/or right prefrontal cortex. The implantation coordinates for the striatum (AP: +1.00, L: 2.5, V: 4.00) and prefrontal cortex PFC (AP: +4.1, L: +1.0, V: −1.2, 20% angle) were determined relative to bregma (Paxinos and Watson, 1998). Cannulae were cemented to the skull using dental acrylic with embedded stainless-steel bone screws.

During the 48 h following surgery, CMA 10 probes (0.5 mm × 2.0 mm or 4.0 mm membrane length with a molecular cutoff 20 000 Da) were inserted into the guide cannulae. The estimated recovery rate was 18–20%. Probes were continuously perfused using a syringe pump CMA/100 (Carnegie Medicine) at a flow rate of 1.0 μl/min with an Mg^{2+}-free Ringer solution containing NaCl 147 mM; KCl 4 mM; $CaCl_2$ 1.2 mM (degassed). A period of 2 h was allowed to establish the basal level of the extracellular catecholamines. Then 30-min dialysate samples were collected with a fraction collector (Bioanalytical Systems). After three baseline samples, the rats were challenged with amphetamine sulfate (RBI), which was dissolved in physiological saline and given subcutaneously at a dose of 1 mg/kg.

Following completion of the experiment, animals were anesthetized with ketamine hydrochloride and acepromazine maleate 1 : 1 mixture (1 μl/g i.m.). Blood samples were obtained via cardiac puncture, and plasma separated for PCP analysis. The rat brain was fixed first with 100 ml of 0.9% saline in 0.1 M phosphate, pH = 7.4, and then with 300 ml of ice-cold 4% paraformaldehyde in 0.1 M phosphate, pH = 7.4. The brains were cryoprotected in 30% sucrose in 0.1 M phosphate. The placement of the probes was determined histologically.

Dopamine Level Determinations

Dopamine levels were determined by high-pressure liquid chromatography with electrochemical detection (HPLC-EC) (BAS-480 system). The dialysate samples (30 μl)—collected in 0.1 N perchloric acid—were injected by autosampler (BAS Sample Sentinel) onto a microbore C_{18} 100 × 2 mm^2 column. The sample was eluted with filtered, degassed mobile phase (NaH_2PO_4 25 mM; sodium citrate 50 mM; disodium-EDTA 27 μM; diethylamine-HCl 10 mM; 1-octanesulfonic acid, sodium salt; methanol 3% v/v; dimethylacetamide 2.1% v/v; pH = 3.5) at a flow rate of 0.4 ml/min, yielding a retention time of 6.0 min. Classic glassy carbon electrodes (BAS) *vs* the Ag/AgCl reference electrode at 0.60 V and sensitivity levels of 0.5 nA were used for dopamine detection.

Data were acquired on an IBM-compatible PC using BAS-5 interface. Standard curves were constructed using 7 pts between 0.625 and 80 pg/10 μl for dopamine. Correlation coefficients (r) of >0.98 were obtained for all curves. The working standard solutions were stored at −80°C and 10 μl of the standard solution was injected between biological samples.

Data Analysis

Primary dependent measures consisted of dopamine levels prior to and following amphetamine administration. Data were analyzed using repeated measures ANOVA with Geisser-Greenhouse correction, with within-subject measure of time following amphetamine injection (ie fraction or observation number) and between-subject factor of drug (PCP or saline). Significant main or interaction effects were

followed up by a between-group *post hoc t*-test. Two-tailed statistics with an α level of significance of $p<0.05$ were used throughout. Data in the text are mean $\pm$ SEM.

RESULTS

3-Month Treatment Study

An initial experiment evaluated the effects of glycine on PCP-induced dopaminergic hyper-reactivity during long-term PCP administration, to mimic the likely effects of prolonged clinical treatment with glycine in humans. This was considered the longest time over which rats could technically be maintained on PCP using osmotic mini-pumps. Rats were treated for 3 months with PCP (5 mg/kg/day) or saline with or without concurrent glycine administration. Glycine was administered orally via an enriched diet (8% glycine by weight). Serum PCP levels obtained at the time of killing, 69.3 $\pm$ 7.0 ng/ml, were within the range associated with PCP psychosis in humans (Javitt and Zukin, 1991) and were not significantly different between glycine-treated and control animals ($p>0.2$).

Glycine treatment led to a significant, two-fold increase in serum levels of glycine from 221 $\pm$ 21 to 480 $\pm$ 97 μM ($t=2.73$, $p<0.02$) and a 1.5-fold increase in serine levels from 167 $\pm$ 10 to 238 $\pm$ 21 μM ($t=3.25$, $p<0.01$). Although significantly elevated *vs* control, these levels are somewhat lower than those achieved during clinical treatment with glycine (Heresco-Levy *et al*, 1999; Javitt *et al*, 2001; Leiderman *et al*, 1996). In order to verify that increased serum levels were associated with increased brain concentrations, amino acid levels were determined from pre-amphetamine microdialysate samples as well. As in serum, microdialysate glycine levels were increased approximately two-fold in glycine-treated *vs* control animals ($p<0.05$). A 1.4-fold increase was observed for microdialysate serine levels, but the degree of between-group difference did not reach statistical significance (Figure 1). Glutamate, aspartate, and glutamine levels were unchanged.

A 3-month treatment with PCP led to a significant 1.5-fold increase in amphetamine-induced PFC dopamine release *vs* control animals (Figure 2), as reflected in a significant group (PCP/control) $\times$ time interaction during the 0–210 min post-amphetamine treatment interval ($F_{9,54}=4.4$, $p=0.006$). In contrast, in animals receiving a glycine-enriched (8% by weight) diet, no PCP $\times$ time interaction was observed ($F_{9,45}=0.1$, $p=0.9$), although the main and interactive effects of glycine were not significant.

In control animals, the effects were most pronounced 150–210 min following amphetamine administration. A *post hoc* analysis was therefore conducted over this period alone. Over this time period, significant glycine $\times$ PCP ($F_{1,12}=5.2$, $p=0.04$) and glycine $\times$ PCP $\times$ time ($F_{2,24}=4.7$, $p=0.02$) interactions were observed.

2-Week, 16% Glycine Diet

As the glycine levels obtained during the 3-month study were somewhat below those obtained during clinical trials, a second study investigated the effects of a higher glycine dose (16% by diet) for 2 weeks. Owing to the shorter treatment duration, a higher dose of PCP was used (15 mg/kg/day) for this study. The mean PCP levels among treated animals were 85.4 $\pm$ 6.6 ng/ml, with no significant difference between animals receiving regular *vs* high-glycine diet. Serum glycine levels in rats receiving 16% glycine diet, 1236.0 $\pm$ 253.4 nmol/ml, were increased five-fold over levels in rats receiving a regular diet, (238.4 $\pm$ 50.7 nmol/ml, $t=3.93$, $p<0.001$) and within the range obtained during clinical studies with glycine (Heresco-Levy *et al*, 1999; Javitt *et al*, 2001; Leiderman *et al*, 1996).

For this study, dopamine levels were measured in both the frontal cortex and striatum (Figure 3). In animals receiving a regular diet (left panels), subchronic PCP treatment led to a significant increase in amphetamine-stimulated DA release in both the PFC and striatum, as reflected in the significant PCP $\times$ time effects (FC: $F_{9,252}=6.9$, $p<0.001$; STR: $F_{9,153}=5.8$, $p<0.001$). These effects were prevented in animals receiving a high-glycine (16% by weight) diet, as reflected in the absence of significant PCP $\times$ time effects ($F_{9,153}=1.3$, $p>0.2$; STR: $F_{9,126}=0.2$, $p>0.9$).

In this data set, PCP treatment led to significant potentiation of amphetamine-stimulated dopamine release throughout the 30–210 min period in both the PFC ($F_{1,43}=13.5$, $p=0.001$) and striatum ($F_{1,33}=4.66$, $p<0.04$). This effect was reversed by glycine treatment as indicated by a significant glycine $\times$ PCP interaction in both

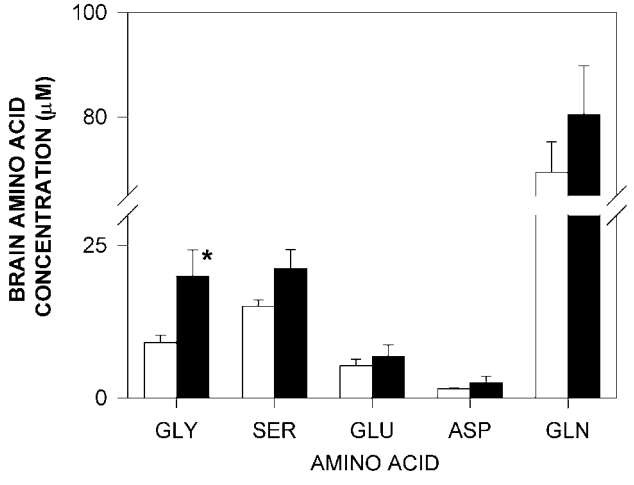

Figure 1 Microdialysis glycine levels in animals receiving regular (open bars, $n=11$) or a high-glycine (8% by weight) diet (solid bars, $n=13$). *$p<0.05$ glycine *vs* control.

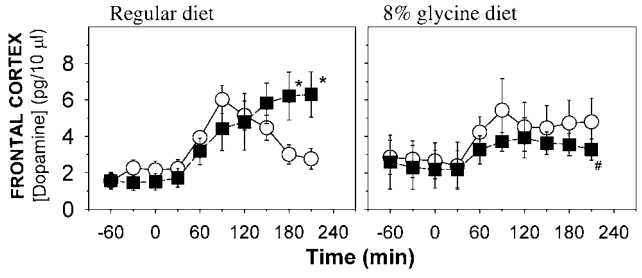

Figure 2 Effect of GLY on AMPH-induced DA release in PFC in animals treated with 5 mg/kg/day PCP for 12 weeks (filled squares) or saline-treated controls (open circles). $n=4$–6 per group. *$p<0.05$ PCP *vs* control, #$p<0.05$ PCP + high-glycine *vs* PCP + regular diet.

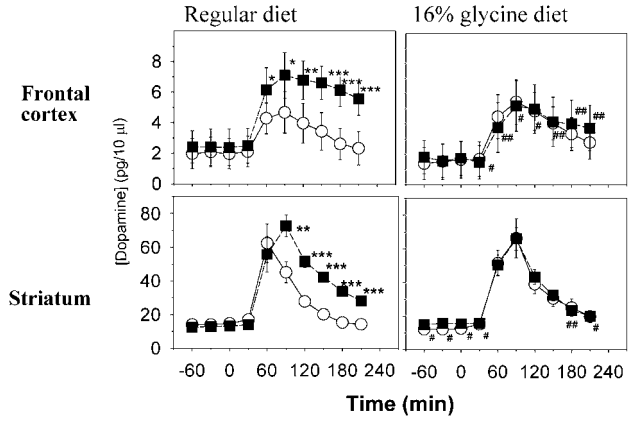

Figure 3 Effect of glycine on amphetamine-induced DA release in PFC (top) and striatum (bottom) in animals treated with 15 mg/kg/day PCP for 2 weeks (filled circles) or saline-treated controls (open circles). $n = 7$–20 per group. $^{*}p < 0.05$ *vs* control, $^{**}p < 0.01$, $^{***}p < 0.001$; $\#p < 0.05$ PCP + high-glycine *vs* PCP + regular diet, $\#\#p < 0.01$.

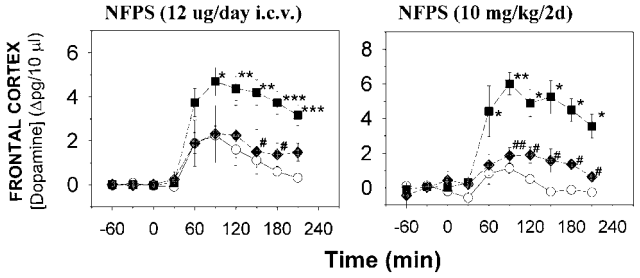

Figure 5 Effect of NFPS on amphetamine-induced DA release following i.c.v (left) or systemic (right) administration. *Left panel*: Amphetamine-induced DA release over basal (−90 to 0 min) in animals receiving NFPS for 2 weeks along with 15 mg/kg/day PCP (shaded circles) *vs* those receiving saline (open circles) or PCP alone (filled squares) $^{*}p < 0.05$ PCP *vs* saline; $^{**}p < 0.01$; $\#p < 0.05$ PCP + NFPS *vs* PCP alone. *Right panel*: amphetamine-induced DA release over the basal level was significantly lower in animals receiving systemic NFPS for 3 days with (shaded diamonds) or without 20 mg/kg/day PCP (open circles) *vs* those receiving PCP alone (filled squares). $^{*}p < 0.05$ PCP *vs* saline; $^{**}p < 0.01$, $^{***}p < 0.001$; $\#p < 0.05$ PCP + NFPS *vs* PCP alone, $\#\#p < 0.01$.

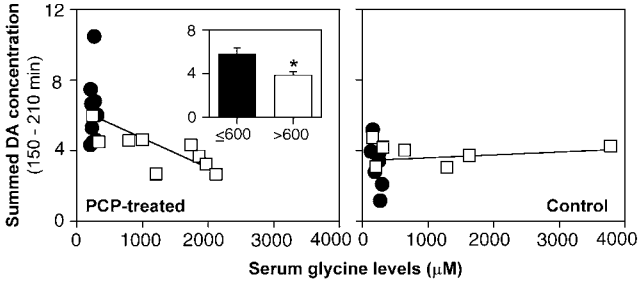

Figure 4 Relationship between serum glycine levels and PFC DA levels during the 150–210 min post-amphetamine-treatment time period in animals receiving PCP (left) and controls (right). Filled symbols indicate animals receiving a regular diet. Open symbols indicate animals receiving a high-glycine diet. Inset Bar chart showing the mean (SEM) PFC DA levels during the 150–210 min period for PCP-treated animals with serum glycine levels $\leqslant 600\,\mu M$ (filled bar, $n = 12$) *vs* animals with the serum glycine levels $> 600\,\mu M$ (open bar, $n = 6$). Regardless of diet, animals with serum glycine levels $\leqslant 600\,\mu M$ showed significantly lower levels of amphetamine-induced DA release than animals with serum glycine levels $> 600\,\mu M$. $^{*}t = 2.91$, $p = 0.01$ $\leqslant 600\,\mu M$ *vs* $> 600\,\mu M$.

brain regions (PFC: $F_{1,43} = 6.58$, $p < 0.02$; STR: $F_{1,33} = 4.93$, $p < 0.04$). For PCP-treated animals, but not controls (Figure 4), there was a significant negative correlation between serum glycine levels and potentiated amphetamine-induced DA release during the 150–210 min period ($r = -0.52$, $p < 0.03$), such that animals with serum glycine levels $> 600\,\mu M$ showed significantly lower levels of amphetamine-induced dopamine release than those with levels $< 600\,\mu M$ ($t = 2.91$, $p = 0.01$).

Glycine Transport Inhibitors

A final series of experiments evaluated the effects of the prototype glycine transport inhibitor NFPS. Owing to limited information concerning its brain penetration, NFPS was administered both intracerebroventricularly (i.c.v.) and systemically in separate studies. For i.c.v. studies, NFPS was administered via an osmotic minipump using a brain perfusion cannula inserted into the lateral ventricle. A dose of 12 µg/day was used, representing the maximum dosage that could be achieved using this delivery system. For systemic studies, NFPS was administered at a dose of 10 mg/kg i.p. every other day (10 mg/kg/2 day). This dose is reported to induce a two-fold increase in brain glycine concentrations (Atkinson *et al*, 2001). A 3-day treatment regimen was used.

The amphetamine-induced DA release over basal was significantly lower in animals receiving NFPS for 2 weeks along with 15 mg/kg/day PCP *vs* those receiving PCP alone ($F_{9,162} = 2.2$, $p < 0.03$), but not significantly different than in animals receiving saline treatment alone ($F_{9,108} = 0.6$, $p > 0.8$). Animals treated with NFPS systemically ($F_{9,45} = 5.5$, $p < 0.001$) also showed significantly reduced amphetamine-induced dopamine release relative to animals that received PCP alone. The differences between animals treated with PCP and those treated with NFPS along with PCP were significant throughout the 90–210 min interval (Figure 5).

DISCUSSION

NMDA antagonists induce dopaminergic hyperactivity similar to that observed in schizophrenia in both human (Kegeles *et al*, 2000) and rodent (Balla *et al*, 2001b, 2003; Miller and Abercrombie, 1996) studies. In schizophrenia, treatment with NMDA co-agonists such as glycine, D-serine, and D-cycloserine produce significant amelioration of treatment-refractory symptoms, including improvement in both negative and positive symptoms (Javitt, 2002). Glycine effects are observed at serum levels of $> 600\,\mu M$ (Heresco-Levy *et al*, 1999; Leiderman *et al*, 1996). The present study demonstrates that glycine, at similar serum levels, reverses PCP-induced dysregulation of dopamine release in both the PFC and striatum, supporting the concepts first that it is effective in potentiating brain NMDA receptor-mediated neurotransmission and, second, that potentiation of NMDA receptor-mediated neurotransmission may be beneficial in schizophrenia.

The present results can be interpreted on both a neurochemical and clinical level. On the neurochemical level, these results are consistent with other studies showing the regulation of frontal and striatal DA systems by NMDA receptors. For example, Svensson (2000) has demonstrated that NMDA antagonists induce alteration in the firing pattern of neurons in the ventral tegmental area projecting to the PFC. Similarly, mice lacking the NR1 subunit show dysregulated dopaminergic systems (Miyamoto *et al*, 2001). We have previously observed that systemic PCP does not potentiate DA release induced by local amphetamine administration (Balla *et al*, 2001a), suggesting circuit level or other interactions. Hippocampal glutamatergic afferents may also regulate firing of midbrain DA neurons via both NMDA and non-NMDA afferents (Floresco *et al*, 2001). Thus, the critical sites of interaction in the present study may not be within the PFC or striatum, but may be within regions such as the ventral tegmental area and substantia nigra that project to these regions. Following single-dose administration, NMDA antagonists such as PCP or MK-801 stimulate DA release for 1–2 h (Javitt *et al*, 1999). In the present study, no sustained elevation in the basal DA levels was observed following long-term administration, suggesting that tolerance develops to this effect during chronic continuous administration.

On a clinical level, the present results may help explain the beneficial effects of NMDA agonists on symptoms of schizophrenia. Initial studies of NMDA agonists focused on the effect of these agents on persistent negative symptoms (Javitt, 2002). More recent studies, however, have documented the effects of these agents even on persistent positive symptoms in patients receiving typical or atypical antipsychotic treatment (Heresco-Levy *et al*, 2003; Tsai *et al*, 1998). Excess dopamine release in striatum has been shown to be associated with increased positive symptoms in amphetamine-challenged schizophrenic patients (Laruelle *et al*, 1996). To the extent that dopaminergic dysregulation in schizophrenia is due to underlying NMDA dysfunction, the ability of NMDA agonists to decrease amphetamine-stimulated DA release is consistent with the clinical therapeutic effect of these agents. In the present study, glycine was administered along with PCP throughout the study and prevented PCP effects. Future studies will be needed to determine the degree to which glycine supplementation added during the course of PCP treatment can reverse already established dopaminergic hyper-reactivity.

In schizophrenia, negative symptoms are thought to reflect functional hypodopaminergia, especially in the prefrontal cortex (Davis *et al*, 1991; Moore *et al*, 1999). However, as yet no studies have demonstrated reduced dopamine levels. In the present study, no change in baseline dopamine levels were observed in the prefrontal cortex. This finding is consistent with observations in both rats (Jentsch *et al*, 1998) and monkeys (Jentsch *et al*, 1997) that tissue dopamine levels remain unchanged following chronic treatment with NMDA antagonists, such as ketamine, PCP, or MK-801, although turnover rates (determined by metabolite:dopamine ratios) increase. The present findings argue against disturbances in absolute extracellular dopamine levels in schizophrenia, although not against disturbances in dopamine turnover. Since NMDA and D1 receptors show mutual facilitatory interactions (eg Chen and Yang, 2002; Scott *et al*, 2002; Wang and O'Donnell, 2001), disturbances in dopaminergic neurotransmission may occur independent of changes in extracellular dopamine levels.

The finding of exaggerated PFC response to amphetamine challenge in PCP-treated animals is also consistent with recent observations in schizophrenia. The majority of amphetamine challenge studies in schizophrenia have been conducted primarily with neuroleptic-treated, stabilized patients. In such patients, amphetamine typically produces small, but consistent improvement in cognitive functioning (eg Daniel *et al*, 1991; Goldberg *et al*, 1991). In contrast, a recent study investigated the effects of the indirect dopamine agonist methylphenidate in both acute and stabilized patients using the Word Production Test (Szeszko *et al*, 1999), a putative marker of prefrontal functioning (Yurgelun-Todd *et al*, 1996). Acute-phase patients showed significantly lower response rates than stabilized patients. Further, both groups showed deterioration of performance during methylphenidate challenge, along with increasing conceptual disorganization. By contrast, methylphenidate typically improves the prefrontal performance to novel tasks in normal volunteers (Elliott *et al*, 1997; Mehta *et al*, 2000), supporting the concept that patients show increased susceptibility to the disorganizing effects of prefrontal hyperdopaminergia following psychostimulant administration.

NMDA antagonists induce working memory dysfunction similar to those observed in schizophrenia (Adler *et al*, 1998; Krystal *et al*, 2000, 1994; Umbricht *et al*, 2000). Further NMDA agonists decrease conceptual disorganization (Javitt, 2002), and potentially improve working memory performance in schizophrenia (Tsai *et al*, 1998). The present findings are thus consistent with a model in which persistent NMDA dysfunction produces a tonic level of working memory dysfunction, which is prone to further disruption by hyperdopaminergia induced by either acute decompensation or psychopharmacological agents.

Aside from demonstrating the effectiveness of glycine and glycine transport inhibitors against PCP-induced augmentation of amphetamine-stimulated dopamine release, the present study provides the first demonstration that administration of clinically relevant glycine doses in animals leads to significant elevation of brain glycine levels. The administration of an 8% glycine diet to rodents, which led to a two-fold increase in serum glycine levels, also led to a two-fold increase in microdialysate glycine levels and a 1.4-fold increase in microdialysate serine levels. As glycine enters the brain by passive diffusion across the blood–brain barrier, serum and brain glycine levels would be expected to equilibrate over time. In acute studies in humans, an 8-fold increase in serum glycine levels produced a two-fold increase in CSF levels 1.5 h after administration (D'Souza *et al*, 2000). Although the glycine elevation observed in that study was significant, the results of the present study suggest that the degree of elevation of brain glycine levels *vs* serum during chronic treatment may be substantially higher than has been observed in acute studies. Glycine was well-tolerated in rats even during 3-month administration, as has been noted previously (Shoham *et al*, 1999). The present study suggests that administering 8–16% by weight glycine-enriched diet may be an effective method for producing

serum glycine levels similar to those observed during clinical studies in humans.

The mean serum PCP level in this study is similar to that observed in other studies using chronic PCP administration (eg Proksch *et al*, 2000). Notably, because of the slow infusion rate used, peak serum concentrations were substantially below those encountered during i.v. (Proksch *et al*, 2000) or i.p. (Bailey and Guba, 1980) administration of even relatively low doses of PCP (eg 1–3 mg/kg). NMDA antagonists, such as PCP, may induce neurodegeneration of structures involved in this study (ie PFC, striatum) following repeated, acute high-dose administration, for example, 20 mg/kg i.v. given once per day for 5 days. Neurotoxic effects, moreover, are significantly more pronounced in female, than male, rats (Johnson *et al*, 1998). Previous studies with male rats using doses similar to those in the present study (eg 10 mg/kg/day) have failed to observe significant effects of PCP on behavioral sensitization (Johnson *et al*, 1998) or PCP receptor binding (Burke *et al*, 1995).

In summary, the present treatment approaches for schizophrenia are based primarily upon dopaminergic models of the disorder. Although many patients respond well to antidopaminergic therapies, the majority of patients show persistent symptomatology despite treatment with either typical or atypical antipsychotic agents. The present study supports clinical research demonstrating significant improvement in negative, positive, and cognitive symptoms of schizophrenia with NMDA agonists including glycine, D-serine, and D-cycloserine (Goff *et al*, 1999; Heresco-Levy *et al*, 1999; Javitt *et al*, 2001, 1994; Shoham *et al*, 2001; Tsai *et al*, 1998). A prior study with the first available glycine transport inhibitor, GDA, demonstrated its ability to reverse PCP-induced hyperactivity in rodents (Javitt *et al*, 1999; Javitt and Frusciante, 1997). This study is the first to examine the *in vivo* effects of a more selective glycine transport inhibitor, NFPS. The finding that glycine transport inhibitors exert *in vivo* effects similar to glycine or other NMDA agonists supports the hypothesis that these agents may be useful in the treatment of persistent symptoms of schizophrenia.

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