Psychopharmacology (2008) 198:363–374
DOI 10.1007/s00213-008-1151-6

ORIGINAL INVESTIGATION

Glycine and D-serine, but not D-cycloserine, attenuate prepulse inhibition deficits induced by NMDA receptor antagonist MK-801

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Received: 3 September 2007 / Accepted: 19 March 2008 / Published online: 24 April 2008

Abstract
Rationale Several agents that stimulate the glycine site of *N*-methyl-D-aspartate (NMDA) receptors have been reported to moderately improve both negative symptoms and cognitive dysfunctions in patients with schizophrenia. However, differences in efficacy have also been reported, and further comparative pharmacological studies are still needed.
Objectives We aimed to explore the effects of two glycine site agonists of the NMDA receptor, glycine and D-serine, and a partial agonist, D-cycloserine, on prepulse inhibition (PPI) deficits induced by a NMDA receptor antagonist, MK-801, in mice. Furthermore, we performed in vivo microdialysis and additional PPI measurements using a selective glycine site antagonist to verify if the beneficial effects observed after the systemic administration of glycine were due to glycine itself via its activity at the glycine site.
Results High doses of glycine (1.6 g/kg) and D-serine (1.8 and 2.7 g/kg) significantly attenuated MK-801-induced PPI deficits. In contrast, D-cycloserine did not show any amelioration of MK-801-induced PPI deficits at doses ranging from 7.5 mg/kg to 60 mg/kg. The selective glycine site antagonist, L-701,324 (10 mg/kg), antagonized the effect of glycine on MK-801-induced PPI deficits. Furthermore, in vivo microdialysis demonstrated that intraperitoneal injection of glycine significantly increased glycine and L-serine levels, but decreased D-serine levels in the prefrontal cortex.
Conclusions The findings of the present study suggest that glycine and D-serine but not D-cycloserine could attenuate PPI deficits associated with NMDA receptor hypofunction via NMDA glycine sites in the brain.

Keywords NMDA receptor · Glycine · D-serine · D-cycloserine · Prepulse inhibition · Microdialysis

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Introduction

Several lines of evidence suggest that hypofunction of *N*-methyl-D-aspartate (NMDA) receptors might be involved in the pathophysiology of schizophrenia (Javitt and Zukin 1991; Goff and Coyle 2001; Hashimoto et al. 2004). Clinical studies demonstrated that open channel blockers of NMDA receptors such as phencyclidine and ketamine elicit psychomimetic effects in both schizophrenic patients and normal subjects (Javitt and Zukin 1991; Adler et al. 1999; Krystal et al. 1994). Furthermore, it has been demonstrated that serum levels of D-serine, an endogenous agonist at NMDA receptors, were decreased in patients with schizophrenia (Hashimoto et al. 2003) and

that cerebrospinal fluid (CSF) levels of D-serine in schizophrenic patients were also lower than those of the controls (Hashimoto et al. 2005; Bendikov et al. 2007), supporting the hypofunction hypothesis of schizophrenia. The NMDA receptor hypofunction hypothesis of schizophrenia suggests that increasing NMDA receptor function via pharmacological manipulation could provide a potential new strategy for the management of schizophrenia. Currently, the glycine modulatory sites on NMDA receptors present the most attractive therapeutic targets for the treatment of schizophrenia (Hashimoto 2006).

It has been reported that glycine and D-serine, both glycine site agonists, and a partial agonist, D-cycloserine, significantly reduce negative symptoms and cognitive dysfunctions in schizophrenia (Heresco-Levy et al. 2004a, 2005; Goff et al. 2005). Moreover, differences in the effects of these compounds have been reported, namely less marked effects have been reported for D-cycloserine than for the others (Tuominen et al. 2005; Heresco-Levy and Javitt 2004b), and the use of D-cycloserine with clozapine worsens negative symptoms (Goff et al. 1999). Therefore, it is of great interest to investigate the mechanisms of actions of these compounds in terms of their effects on both NMDA receptors and clinical symptoms.

Prepulse inhibition (PPI) of the startle reflex, used in the current study, is an established paradigm for the assessment of sensorimotor gating systems, which are disturbed in schizophrenia (Swerdlow et al. 1994; Braff et al. 2001; Geyer et al. 2002). In animal models, NMDA receptors have been shown to play a crucial role in a circuit involved in PPI (Geyer et al. 2001). Several studies reported that the deficits in PPI related to the NMDA receptors were reversed by antipsychotic drugs (Swerdlow et al. 1994; Yamada et al. 1999), although the results were inconsistent. Therefore, the impairments of PPI induced by NMDA receptor antagonists have been used as an animal model of schizophrenia for developing potential therapeutic drugs. Recently, it was reported that D-serine had tendency to ameliorate PPI deficits induced by the NMDA receptor antagonist MK-801 in mice, although its reversal effect did not reach statistical significance (Lipina et al. 2005). To the best of our knowledge, neither glycine nor D-cycloserine has been examined in terms of its effects on PPI deficits in animal models of NMDA receptor hypofunction.

To account for the differences in the effects of glycine site agonists on NMDA receptors, we examined the effects of these agonists on PPI deficits induced by MK-801. While D-serine and D-cycloserine have high affinity and selectivity for glycine sites of NMDA receptors, glycine is known to have high affinity for inhibitory glycine_A receptors, which are distributed primarily in spinal cord and pons (Lynch 2004). Under moderate doses, glycine shows low blood–brain barrier (BBB) permeability (Oldendorph 1971; Toth and Lajtha 1986; D'Souza et al. 1995). Furthermore, several studies of both rats and subjects with schizophrenia have suggested that the administration of glycine led to increases in the concentration of D-serine in the central nervous system (CNS) (Takahashi et al. 1997; Heresco-Levy et al. 2004a). Therefore, a secondary purpose of the present study is to examine whether glycine or D-serine in the brain could be increased following systemic injection of glycine.

Materials and methods

Subjects

Animals

Eight-week-old ddY male mice were purchased from Nihon SLC (Hamamatsu, Shizuoka, Japan). The ddY mice were developed in Germany and introduced to the National Institute of Infectious Diseases in Japan in 1963. These mice were not in genetic homogeneous, but the ddY closed colony is most often used to support research in many areas in Japan. The animals were kept for at least 1 week in the animal colony at our laboratory before the beginning of behavioral testing. The mice were housed five to six per cage and kept at a controlled temperature (23°C±1°C) and on a 12-h light/dark cycle (light on at 0700 hours). The animals were provided food and water ad libitum. All behavioral testing was conducted between 0900 and 1700 hours. Mice were randomized with regard to day and treatment, and were only used once. The research and animal care were carried out according to the Guide for Animal Experimentation of the Chiba University Graduate School of Medicine.

Drugs

Glycine (Wako Pure Chemical Industries, Osaka, Japan), D-serine (Sigma-Aldrich, Steinheim, Germany), D-cycloserine (Meiji Pharmaceutical Ltd., Tokyo, Japan), and MK-801 (Sigma-Aldrich) were dissolved in physiological saline. A selective antagonist of the glycine site of NMDA receptors, L-701,324 (7-chloro-4-hydroxy-3-[3-phenoxy] phenylquinolin-2[1H]-one; Sigma-Aldrich), was dissolved in 25% polyethylene glycol (PEG300; Wako Pure Chemical Industries) with pH adjusted to 10 with 1 M NaOH, according to the previous reports (Bristow et al. 1996a, b; Obrenovitch and Zilkha 1996, 1997). All chemicals were injected intraperitoneally (i.p.) into the animals at a volume of 10 mg/kg.

Apparatus and procedures

Prepulse inhibition

The mice were tested to assess their acoustic startle reactivity in two startle chambers (SR-LAB, San Diego Instruments, CA, USA) using standard methods described by Swerdlow and Geyer (1998). Background noise was set at 65 dB. Four trial types were used. Pulse-alone trials (P) consisted of a single white-noise burst (120 dB, 40 ms). The prepulse + pulse trials (PP69P, PP73P, PP77P, and PP81P) consisted of a prepulse of noise (20 ms at 69, 73, 77, or 81 dB, respectively), which was followed 100 ms after the prepulse onset by a startle pulse (120 dB, 40 ms). No-stimulus (NS) trials consisted of the background noise only. Sessions were structured as follows: (1) 15-min acclimation at background noise level; (2) five P trials; (3) ten blocks, each containing all ten trials (P, PP69P, PP73P, PP77P, PP81P, and NS) in pseudorandom order; and (4) five P trials. Intertrial intervals were distributed between 7 and 23 s. The average percent reduction in startle intensity between pulse and prepulse + pulse trials at all four prepulse levels was defined as the PPI level. The percentage PPI induced by each prepulse intensity was calculated as follows: [1-(startle amplitude on prepulse trial)/(startle amplitude on pulse alone)] × 100%. Startle magnitude in this formula was calculated as the average response to all of the P trials, excluding the first and last blocks of five P trials.

In vivo microdialysis

Mice were anesthetized with sodium pentobarbital before the stereotactic implantation of a probe into the left prefrontal cortex (+1.5 mm anteroposterior, +0.5 mm mediolateral from the bregma, and −1.5 mm dorsoventral with respect to the dura). Probes (D-I-2-01; implantation depth = 2 mm, length of dialysis membrane = 1 mm, Eicom, Kyoto, Japan) were secured onto the skull using stainless-steel screws and dental acrylic. Twenty-four hours after surgery, in vivo microdialysis was performed on conscious mice. Probes were perfused continuously with artificial CSF (147 mM NaCl, 4 mM KCl, and 2.3 mM $CaCl_2$) at a rate of 2 μl/min. A 1.6-g/kg glycine was injected intraperitoneally at 0 min, and the dialysate was collected from −90 to 210 min in every 30-min fraction.

High-performance liquid chromatography assessments

Measurement of glycine, total serine, and D-/L-serine levels was carried out according to a column-switching high-performance liquid chromatography (HPLC) system (Shimadzu Corporation, Kyoto, Japan), as described previously (Fukushima et al. 2004; Yamada et al. 2005). A 20-μl aliquot of sample was added to 20 μl of.1 M borate buffer (pH 8.0) and 60 μl of 50 mmol/ml 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F; Tokyo Kasei Kyogo, Japan) in CH_3CN. The reaction mixture was then heated at 60°C for 1 min and immediately supplemented with 100 μl of H_2O/CH_3CN(90/10) containing 0.1% trifluoroacetic acid (TFA) to stop the reaction. A 10-μl aliquot of the resultant solution was injected into the HPLC system. A reversed-phase octadecylsilica (ODS) column (TSKgel ODS-80Ts, Tosoh Corporation, Tokyo, Japan, as column 1) was used for the separation and quantification of total (D- and L-) serine, and the gradient elution of the mobile phase was maintained at a constant flow of 0.8 ml/min. Mobile phase 1a consisted of H_2O/CH_3CN(90/10) containing 0.1% TFA, and phases 1b and c consisted of H_2O/CH_3CN(10/90) containing 0.1% TFA and CH_3CN, respectively. The time program for gradient elution was as follows: 0–25 min, 1a/1b/1c = 92:8:0; 25–35 min, 1a/1b/1c = 0:100:0; and 35–45 min, 1a/1b/1c = 0:0:100. The chiral column (column 2) used for the separation and quantification of D- and L-serine with NBD-F comprised two Sumichiral OA-2500 columns (S; Sumika Chemical Analysis Service, Osaka, Japan), which were connected in tandem. The mobile phase was 15 mmol/ml citric acid in MeOH. The flow rate was isocratically pumped at 0.8 ml/min. The column temperature of all columns was maintained at 35°C. Fluorescence detection was performed at 530 nm with an excitation wavelength at 470 nm.

PPI experimental design and statistical analysis

PPI protocol

Experiment 1 tested the effects of glycine (0.8 and 1.6 g/kg), D-serine (0.6, 0.9, 1.8, and 2.7 g/kg), and D-cycloserine (7.5, 15, 30, and 60 mg/kg), as well as that of MK-801 (0.10 and 0.15 mg/kg), on PPI and startle amplitude. The doses of these compounds were chosen based on previous PPI studies (Le pen et al. 2003; Lipina et al. 2005; Curzon and Decker 1998, Yee et al. 2004), other behavioral data (Nilsson et al. 1997; Karcz-Kubicha et al. 1999; Javitt et al. 1999; Kato et al. 2001), and our preliminary data.

Experiment 2 tested the effects of glycine (0.8 and 1.6 g/kg), D-serine (0.6, 0.9, 1.8, and 2.7 g/kg), and D-cycloserine (7.5, 15, 30, and 60 mg/kg) at doses chosen on the basis of the results of experiment 1, i.e., on disrupted PPI induced by 0.15 mg/kg MK-801.

Experiment 3 tested the antagonistic effects of L-701,324 on PPI reversed by glycine (experiment 2). First, L-701,324 (10 mg/kg) dissolved in PEG300 were examined in terms of its effects on PPI and startle amplitude. This dose was chosen based on the PPI data and the results of

other behavioral studies (Popik et al. 2000; Bristow et al. 1995, 1996a, b). After we found no statistical effects of L-701,324 on PPI and startle magnitude, we examined whether or not L-701,324 could antagonize glycine-improved PPI that had been disrupted by MK-801. This experimental protocol was as follows: L-701,324 was injected i.p. at −45 min, then glycine was injected i.p. at −30 min, and MK-801 was then injected i.p. at −15 min before the onset of acclimation time (0 min).

Data analysis

The percentage of inhibition of startle and basic startle response for different trials was analyzed by two-way analysis of variance (ANOVA) with the Geisser–Greenhouse correction, where drug group was included as a between-subject factor and prepulse intensity as a repeated measurement factor. In experiment 3 on L-701,324, repeated measurement three-way ANOVA was performed with injected compounds as a between-subject factor (L-701,324 vs vehicle, glycine vs vehicle, and MK-801 vs vehicle) and prepulse intensity as a repeated measurement within-subject factor. Bonferroni's correction was used for post hoc comparisons when ANOVA revealed statistically significant differences between the drug groups.

In vivo microdialysis data were analyzed using repeated measures ANOVA with the Greisser–Greenhouse correction and a within-subject factor of time following glycine injection (i.e., fraction of time) and a between-subject factor of drug administered (i.e., glycine or saline). Significant main or interaction effects were followed up by a between-group post hoc t test. The α level of significance for all statistics was $p<0.05$. Data in the text are mean ± SEM.

Results

Prepulse inhibition

Experiment 1: effects of glycine, D-serine, D-cycloserine, and MK-801 on startle magnitude and prepulse intensity

As regards glycine, D-serine, and D-cycloserine, the ANOVAs revealed that these compounds had no effects on either startle amplitude or prepulse intensity at any of the doses used (data not shown). Although MK-801 was not found to exert any significant effects on startle magnitude, the ANOVA revealed the main effects of prepulse and treatment, and no significant interaction between prepulse × treatment (data not shown). Individual comparisons revealed a significant main effect of MK-801 on all prepulse intensities (data not shown). The post hoc Bonferroni's test revealed that at the 0.15-mg/kg dose, MK-801 had a statistically significant effects on the all prepulse intensities (PP69, $p<0.05$; PP73,77,81, $p<0.01$), but at the 0.10-mg/kg dose, a significant effect was observed only on PP77 ($p<0.05$; Fig. 1).

Experiment 2: effects of glycine, D-serine, and D-cycloserine on PPI disrupted by MK-801

Here, we chose doses of glycine, D-serine, and D-cycloserine that were found to have no pretreatment effect on either startle response or any prepulse intensity based on the results obtained in experiment 1.

(a) Glycine

In startle magnitude (Fig. 2, inset), ANOVA revealed significant differences among the four drug groups ($F_{3,47}$= 8.724, $p<0.001$). The post hoc Bonferroni's test revealed that startle magnitudes were significantly higher in the vehicle + MK-801 group ($p<0.01$) and both glycine +MK-801 groups (0.8 g/kg; $p<0.05$, 1.6 g/kg; $p<0.001$) than in the vehicle + vehicle group.

In the analysis of the effects of glycine on each prepulse intensity (Fig. 2), the ANOVA indicated significant differences among drug-treatment groups ($F_{3,42}$=21.145, $p<$ 0.001). The post hoc Bonferroni's test revealed that the prepulse intensities in vehicle + MK-801 group were significantly lower than those in the vehicle + vehicle group ($p<0.001$). Moreover, the prepulse intensities in the glycine 0.8 g/kg + MK-801 group were not significantly different from those in the vehicle + MK-801 group ($p>0.05$), but the prepulse intensities in the glycine 1.6 g/kg + MK-801 group were significantly higher than those in the vehicle + MK-801 group ($p<0.01$). Therefore, the present findings suggested that glycine at a dose of 1.6 g/kg led to an improvement of prepulse intensities disrupted by MK-801.

(b) D-serine

In startle magnitude (Fig. 3, inset), ANOVA showed significant differences among the six drug groups ($F_{5,66}$= 3.282, $p<0.01$). The post hoc Bonferroni's test showed that startle magnitude in the vehicle + MK-801 group was significantly higher than that of the vehicle + vehicle group ($p<0.01$).

In the analysis of the effects of D-serine on each prepulse intensity (Fig. 3), ANOVA revealed significant differences among the drug-treatment groups ($F_{5,61}$=16.992, $p<0.001$). The post hoc Bonferroni's test showed that prepulse intensities in the vehicle + MK-801 group were significantly lower than those of the vehicle-treated group ($p<$ 0.001), and the prepulse intensities in the two high-dose D-serine-treated groups (1.8 g/kg; $p<0.05$, 2.7 g/kg; $p<0.01$)

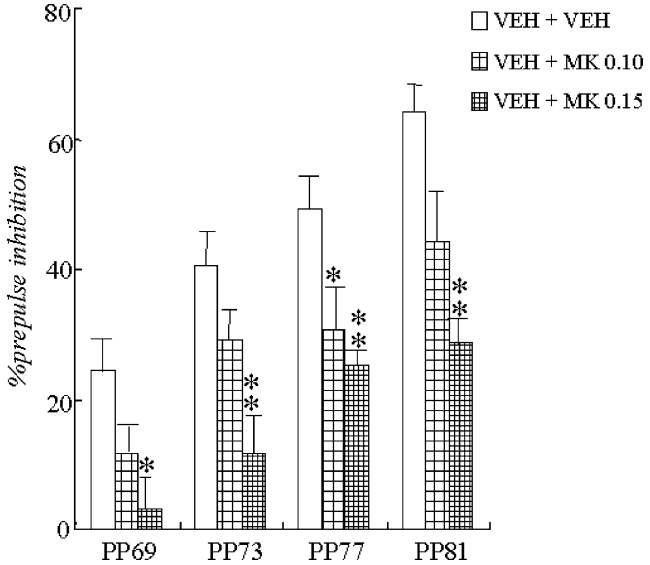

Fig. 1 Effect of MK-801 on prepulse intensities. *$p<0.05$, **$p<0.01$ in comparison with the vehicle-treated group (post hoc Bonferroni's test, ANOVA); N=12~13 per group; *VEH* vehicle, *MK* MK-801

were significantly higher than those in the vehicle + MK-801 group.

(c) D-cycloserine

With respect to startle magnitude (Fig. 4, inset), ANOVA revealed significant differences among the six drug groups ($F_{5,70}$=6.653, $p<0.01$). Results of the post hoc Bonferroni's test revealed that startle magnitudes were significantly higher in the vehicle + MK-801 and D-cycloserine (all doses) +MK-801 groups than in the vehicle-treated group ($p<0.05$).

In the analysis of the effects of D-cycloserine on each prepulse intensities (Fig. 4), ANOVA revealed significant differences among drug-treatment groups ($F_{5,65}$=14.920, $p<0.001$). The post hoc Bonferroni's test indicated that prepulse intensities in the MK-801-treated groups were lower than those in the vehicle-treated group ($p<0.001$), but the prepulse intensities in the D-cycloserine groups were not significantly different from those of the MK-801-treated group.

Experiment 3: effects of a glycine-binding site antagonist, L-701,324, on glycine-induced improved PPI that have been disrupted by MK-801

We also examined whether or not a selective glycine-site antagonists, L-701,324, blocked the amelioration of PPI after injection of glycine. There was a significant main effect of glycine ($F_{1,58}$=10.797, $p<0.01$) and of MK-801 ($F_{1,58}$=10.972, $p<0.01$) but no main effect of L-701,324 ($p>0.05$) on startle magnitude. There were no significant interactions between L-701,324 × glycine × MK-801, L-701,324 × glycine, and between L-701,324 × MK-801. The post hoc Bonferroni's test (Fig. 5, inset) showed no significant differences between drug groups with L-701,324 vs without L-701,324 ($p>0.05$). These results indicated that L-701,324 did not have a significant effect on startle magnitude.

In the analysis of treatment drugs on each prepulse intensity (Fig. 5), there was a main effect of glycine ($F_{1,58}$= 4.645, $p<0.05$) and of MK-801 ($F_{1,58}$=42.815, $p<0.001$)

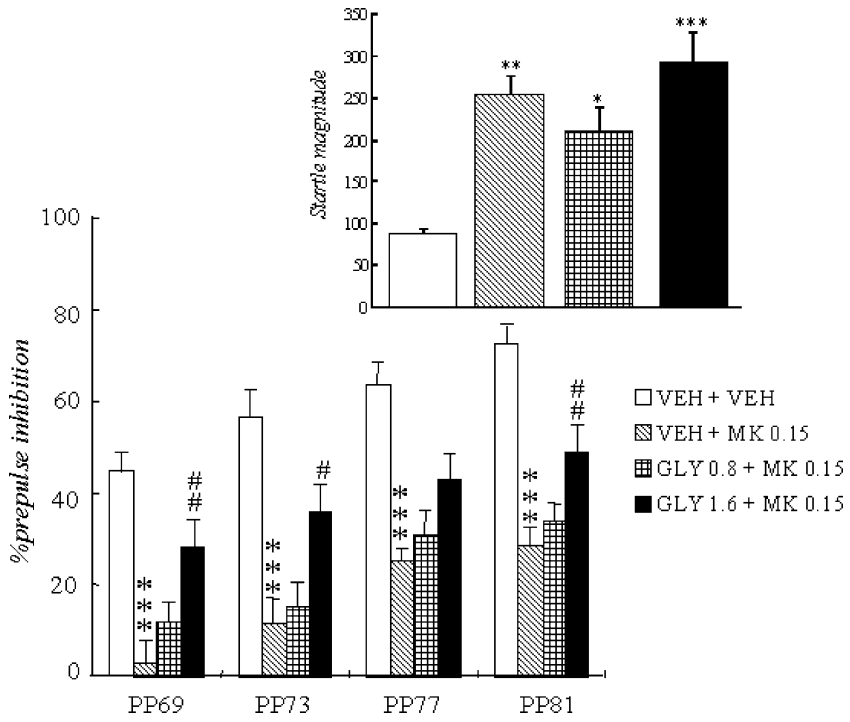

Fig. 2 Effect of glycine on startle magnitude (*inset*) and prepulse intensities disrupted by MK-801. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ in comparison with the vehicle-treated group; #$p<0.05$, ##$p<0.01$ in comparison with the vehicle + MK-801 group (post hoc Bonferroni's test, ANOVA); N=11~12 per group; *VEH* vehicle, *MK* MK-801, *GLY* glycine

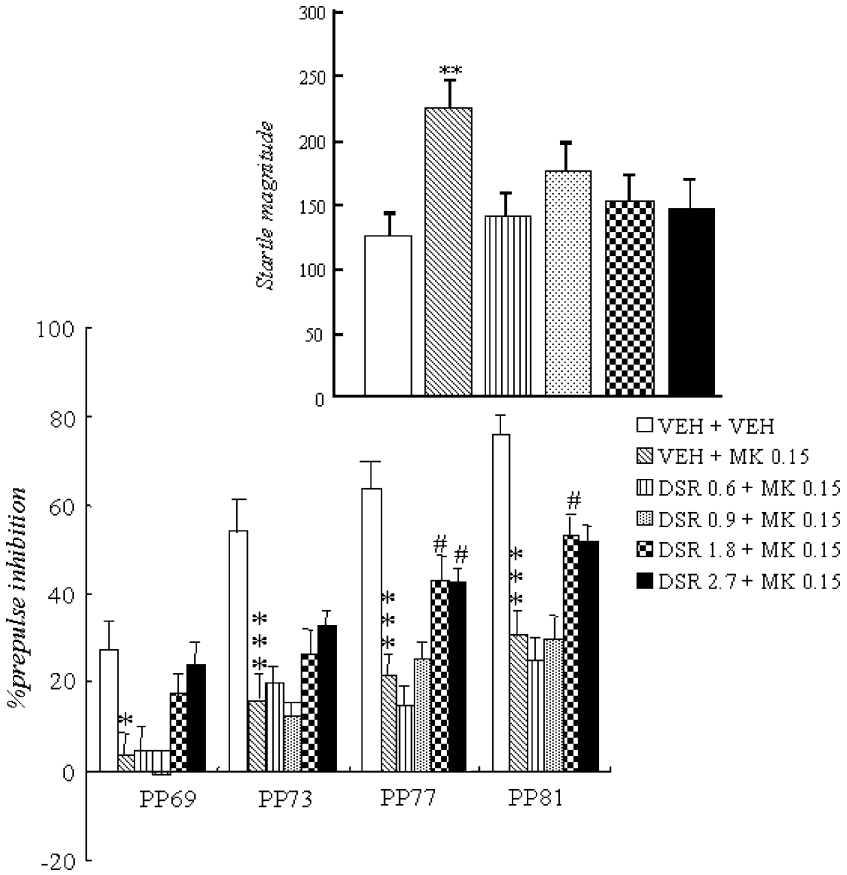

Fig. 3 Effect of D-serine on startle magnitude (*inset*) and prepulse intensities disrupted by MK-801. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ in comparison with the vehicle-treated group; #$p<0.05$ in comparison with the vehicle + MK-801 group (post hoc Bonferroni's test, ANOVA); N=10~12 per group; *VEH* vehicle, *MK* MK-801, *DSR* D-serine

on prepulse intensity but no main effect of L-701,324 ($F_{1,58}$=0.994, $p>0.05$). There was a significant effect of prepulse intensity, reflecting increased prepulse intensity in the presence of greater prepulse intensities ($F_{3,174}$=57.951, $p<0.001$). There was also a significant interaction between prepulse intensity × L-701,324 × MK-801 ($F_{3,174}$=7.019, $p<0.001$).

As there were the interactions between prepulse intensity and drug treatment, % PPI values were analyzed by three-way ANOVA at each level of prepulse intensity. There was a significant interaction between L-701,324 × glycine × MK-801 ($F_{1,58}$=4.083, $p<0.05$) or L-701,324 × MK-801 ($F_{1,58}$=5.849, $p<0.05$) only at PP81. Individual comparisons followed by Bonferroni's test between vehicle + vehicle + MK-801 group and vehicle + glycine + MK-801 group at each level of prepulse intensity revealed significant differences at PP73, PP77 (both $p<0.05$), and PP81 ($p<0.01$), indicating the result similar to experiment 2a (Fig. 2). Additionally, individual comparisons between L-701,324 + glycine + MK-801 group and vehicle + glycine + MK-801 group revealed significant differences in PPI at 77 dB ($p<0.05$) and 81 dB ($p<0.01$; Fig. 5). These results indicated that L-701,324 partially inhibited the effect of glycine on MK-801-induced PPI impairments.

In vivo microdialysis

To investigate the findings that glycine itself reversed PPI deficits that have been induced by MK-801, we also measured glycine and L-/D-serine concentrations in the mouse CNS after the systemic administration of glycine at a dose of 1.6 g/kg using in vivo microdialysis. Glycine concentrations in the CNS were found to be significantly increased (approximately 20-fold increase at maximum) after the systemic administration of glycine, as demonstrated by ANOVA, which showed a main effect of the drug ($F_{1,10}$=91.125, $p<0.001$) and an interaction between drug × time ($F_{7,70}$=63.305, $p<0.001$; Fig. 6a). As regards the L-serine concentration ratio, the ANOVA results indicated a main effect of drug ($F_{1,9}$=7.777, $p<0.05$), whereas no interaction was observed for drug × time ($F_{7,63}$=1.050, $p>0.05$). The post hoc t test revealed significant, approximately 1.5-fold, increases in the L-serine ratio in the glycine-injected group from 60 to 90 min, as compared with the results for the saline-injected group (Fig. 6b). The ANOVA of D-serine indicated that a main effect of drug ($F_{1,8}$=11.362, $p<0.05$) and an interaction between drug × time ($F_{7,56}$=2.576, $p<0.05$). The post hoc t test revealed significant, approximately 0.6-fold, decreases in the D-serine ratio in the glycine-injected group from 120 to

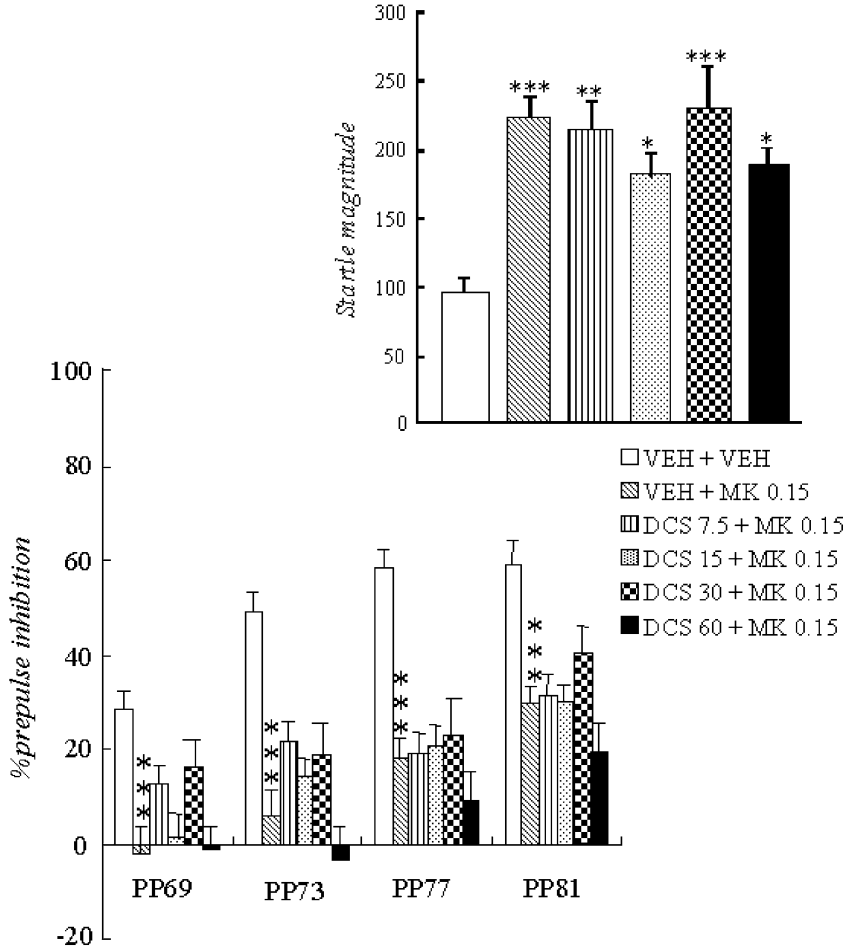

Fig. 4 Effect of D-cycloserine on startle magnitude (*inset*) and prepulse intensities disrupted by MK-801. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ in comparison with the vehicle-treated group (post hoc Bonferroni's test, ANOVA); $N=11\sim12$ per group; *VEH* vehicle, *MK* MK-801, *DCS* D-cycloserine

180 min, as compared with the results for the saline-injected group (Fig. 6c).

Discussion

In the current study, high doses of glycine and D-serine but not D-cycloserine showed similar potency with respect to reversing the PPI deficits induced by MK-801. This is the first study to demonstrate a direct effect of exogenous glycine on MK-801-induced PPI deficits via the glycine sites of NMDA receptors, based on findings as follows: First, using in vivo microdialysis, we found that exogenously injected glycine reached the CNS. Second, we found that a glycine site-selective antagonist, L-701,324, partially antagonized glycine-induced amelioration of MK-801-induced PPI deficits in mice.

In the present study, similar to glycine, D-serine was shown to reverse MK-801-induced deficits in PPI, consistent with a previous report by Lipina et al. (2005). As regards the effectiveness of glycine at reversing PPI deficits, Le Pen et al. (2003) reported that 1.6 g/kg of i.p.-injected glycine was associated with significant improvements in hippocampal-lesioned neonatal rats. However, unlike the present study, their study did not demonstrate that glycine itself, when injected systemically, led to amelioration of PPI deficits via NMDA receptor-direct mechanism. On the other hand, systemic treatment with D-cycloserine, a partial agonist at glycine sites, did not lead to reversal of the deficits in PPI; to the best of our knowledge, this is a novel finding of the present study. Our present results are suggestive of differences between full agonists and a partial agonist in terms of their respective effects on PPI deficits related to NMDA receptor hypofunction.

In this study, both glycine and D-serine were found to have almost similar potency with respect to reversing MK-801-induced PPI deficits. The results are explained by positing a mechanism in which the exogenous agonists, as well as endogenous agonists, can act on glycine sites and, in turn, enhance the activity of receptors. This putative mechanism is consistent with both in vivo and in vitro findings, suggesting that glycine levels did not reach saturation in the synaptic clefts (Danysz and Parsons 1998; Millan 2005). On the other hand, D-cycloserine did not exert any significant effects on MK-801-induced PPI deficits. The dose–response curve of D-cycloserine obtained

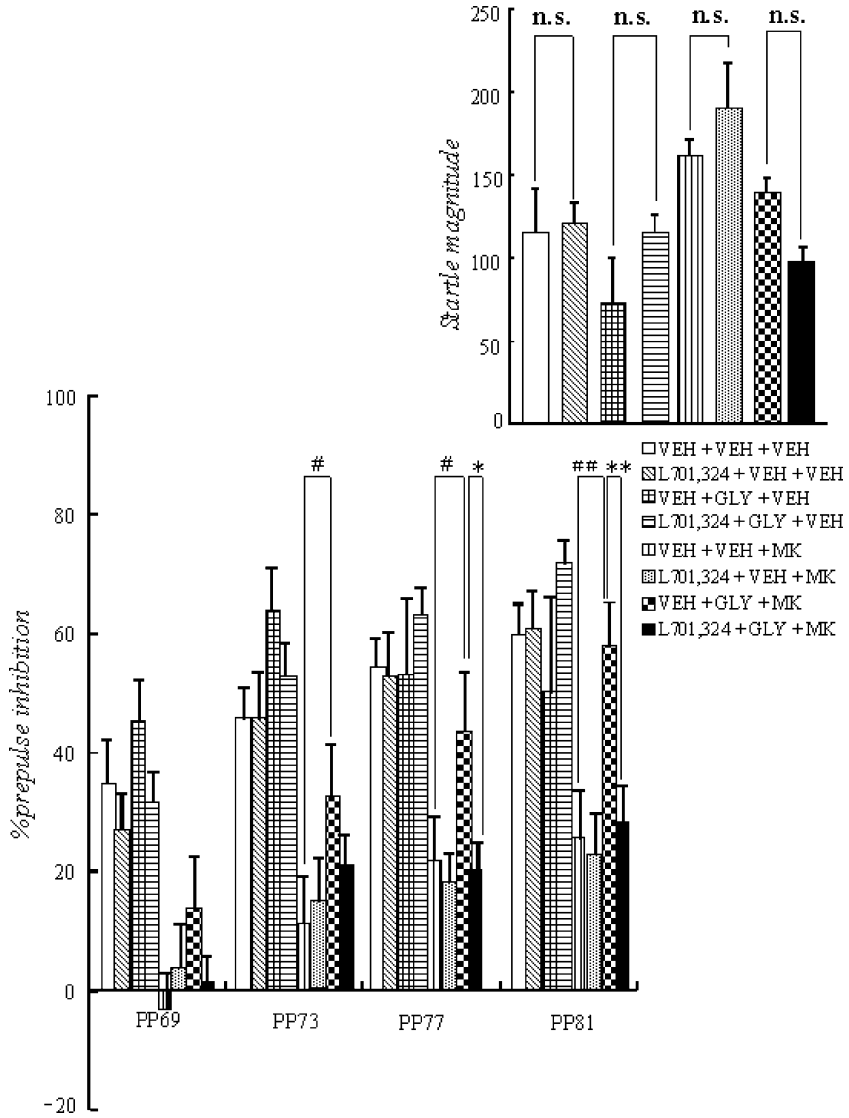

Fig. 5 L-701,324 block glycine-reversed PPI deficits by MK-801 (*inset*) startle magnitude represented by eight drug groups. Four comparisons between with or without L-701,324 in vehicle + vehicle, glycine + vehicle, vehicle + MK-801, and glycine + MK-801 show no significant differences (minimum p value=0.11 in comparison between PEG + glycine + MK-801 and L-701,324 + glycine + MK-801). #$p<0.05$, ##$p<0.01$ in comparison between the vehicle + glycine + MK-801 group and the vehicle + vehicle + MK-801 group; *$p<0.05$ and **$p<0.01$ in comparison between the L-701,324 + glycine + MK-801 group and the vehicle + glycine + MK-801 group; N= 7~11 per group; *n.s.* not significance, *VEH* vehicle, *GLY* glycine, *MK* MK-801

in this experiment was bell-shaped, indicating that D-cycloserine acts as an agonist at low doses (15~30 mg/kg) but an antagonist at higher doses (60 mg/kg), which is in agreement with the results presented in a previous report (Lanthorn 1994). D-Cycloserine alone was observed to have no effect on any prepulse intensity at doses below 30 mg/kg, but diminished effects were seen at higher doses (50~200 mg/kg) in rats (Depoortere et al. 1999). In experiment 1 in our study, D-cycloserine exhibited a similar tendency, i.e., attenuation of each prepulse intensity at a dose of 60 mg/kg, but this finding was not significant (data not shown); doses below 30 mg/kg have no effect. Therefore, the present results suggest that the effects of D-cycloserine on PPI are inferior to those of either D-serine or glycine, and even the peak agonistic effects of D-cycloserine on PPI do not reach significant level. These findings might support the clinical findings of augmentation therapy by the glycine-site agonists at the NMDA receptors added on ongoing antipsychotics for schizophrenia, in which the effect size of D-cycloserine for negative symptoms was slightly smaller than that of either glycine or D-serine (Tuominen et al. 2005). Observed differences in the clinical benefits achieved with these compounds may be related to differences in the potency required of the two full agonists, glycine and D-serine, and a partial agonist D-cycloserine to bring about a reversal of PPI deficits induced by NMDA receptor antagonists.

In the clinical trials for schizophrenia, the dose of glycine for human was 0.4–0.8 g/kg per day, whereas that of D-serine was 30 mg/kg per day (Touminen et al. 2005). The pharmacological reasons of the high-dose setting of glycine in treatment for schizophrenia are based on the findings that low dose of glycine does not penetrate the BBB (Oldendorph 1971; Toth and Lajtha 1986; D'Souza et al. 1995) and that glycine may have lower affinity at the glycine site of the NMDA receptor than D-serine by several

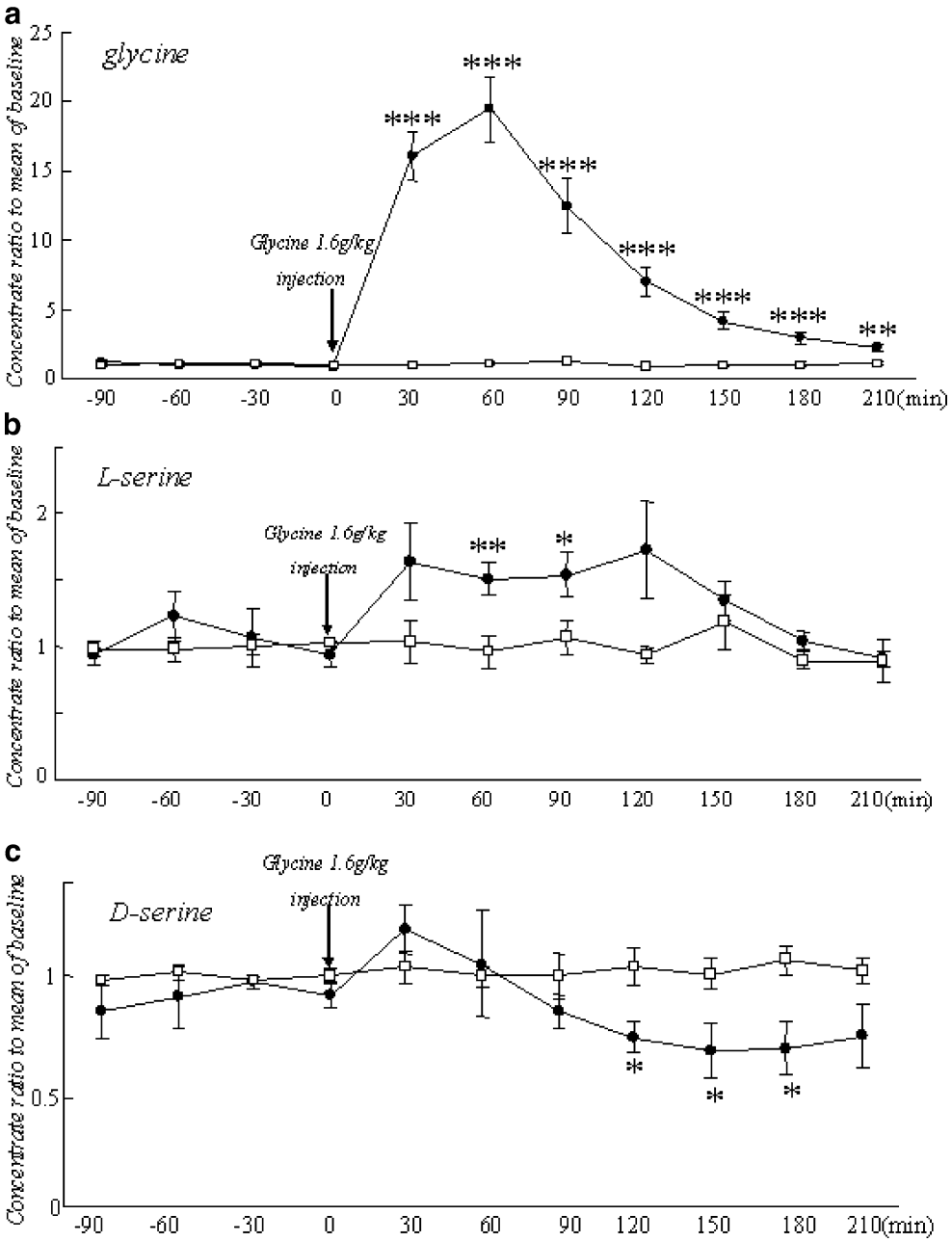

Fig. 6 **a** Effects of intraperitoneal glycine injection (1.6 g/kg) on glycine concentrate ratio in the CNS. **b** Effects of intraperitoneal glycine injection (1.6 g/kg) on L-serine concentrate ratio in the CNS. **c** Effects of intraperitoneal glycine injection (1.6 g/kg) on D-serine concentrate ratio in the CNS. The amino acids ratio (**a** glycine, **b** L-serine, **c** D-serine) of the glycine-injected group is represented by *filled circles* and that of the saline-injected group by *open squares*; ***$p<0.001$, **$p<0.01$, and *$p<0.05$, glycine-injected group vs saline-injected group; N=5~7 per group

previous reports (Kemp and Leeson 1993; Schell et al. 1995). In human studies, D-serine has been successfully used at doses 15- to 25-fold lower than those of glycine. However, our results demonstrated that approximately equal doses of D-serine (1.8 and 2.7 g/kg) were required to reverse PPI, relative to glycine (1.6 g/kg) in mice. A previous report using C57BL/6J mice by Lipina et al. (2005) strongly supported our data, showing that a relative high dose (0.9 g/kg) of D-serine expressed tendency of PPI reversal. These data in mice suggest that high dose of D-serine is needed for PPI improvement in a single systemic administration challenge. It is difficult to compare between the doses for single injection to reverse PPI in an animal model and those for subchronic administration to treat negative symptoms in clinical trials for augmentation of antipsychotic treatment. However, it is important to investigate how much single dose of glycine or D-serine can reverse PPI in patients with schizophrenia in the near future. Moreover, it will be necessary to examine whether subchronic treatment of high-dose D-serine may be more effective than that of low-dose D-serine for improvement of symptoms in patients with schizophrenia.

In the same way as the doses of D-serine, there were other differences about PPI phenomenon between rodents and humans. Our results in mice showed that glycine treatment reversed PPI deficits by the NMDA receptor

antagonist, MK-801. In contrast to an animal model, Heresco-Levy et al. (2007) reported that high glycine levels are associated with PPI deficits in chronic schizophrenia patients. In addition, several studies reported that ketamine, the NMDA receptor antagonist, increased PPI in human subjects (Mansbach 1991; Johansson et al. 1995; Duncun et al. 2001; Abel et al. 2003). These findings about human PPI studies were inconsistent with those about animals. The different results might be explained by the different dose ranges of ketamine (0.5 mg/kg for humans vs 5.6 to 36 mg/kg for animals) or by the different conditions (chronic schizophrenia for human study vs acute glycine treatment for an animal model), respectively. On the other hand, another explanatory reason might be that the relevance of NMDAR-mediated neurotransmission for the PPI phenomenon may be different in humans vs rodents, as discussed by Duncun et al. (2001).

Several studies suggested that the administration of glycine leads to subsequent increases in D-serine (Takahashi et al. 1997; Heresco-Levy et al. 2004a). We therefore examined the concentrations of glycine and L-/D-serine in the CNS using in vivo microdialysis to determine whether the amelioration of MK-801-induced PPI deficits by glycine was due to glycine itself or D-serine. The results showed that glycine reached the peak levels (20-fold baseline levels) in the CNS concentration approximately 60 min after injection; almost concurrently, L-serine increased to approximately 1.5-fold baseline levels. On the other hand, the D-serine concentration decreased slowly (0.7-fold baseline) within a time delay of 120 to 180 min later than the changes observed in the case of glycine and L-serine. At approximately 210 min after glycine injection, the levels of all compounds reversed to baseline levels. Alterations in L-/D-serine concentrations in the extracellular spaces could reasonably be explained as follows: Inside the astroglia, glycine may be converted into L-serine by the glycine cleavage system and serine hydroxymethyltransferase (Verleysdonk et al. 1999), which would in turn lead to increases in L-serine. As regards D-serine, recent in vitro studies have shown that glycine inhibits the activity of serine racemase, which synthesize directly from L-serine into D-serine in astroglia (Wolosker et al. 1999a, b; Dunlop and Neidle 2005; Strisovsky et al. 2005). Therefore, exogenous glycine treatment may induce decreases in endogenous D-serine via an inhibition of serine racemase in adults. This explanation assumes alterations in L-/D-serine, and because the mechanisms regulating glycine and L-/D-serine concentrations remain unclear, they should be investigated in the future studies.

In conclusion, this was the first study to demonstrate that systemic administration of high-dose glycine led to the amelioration of MK-801-induced PPI deficits; the mechanism appears to directly involve the glycine sites of NMDA receptors accessed through the BBB. Moreover, the size effect was very similar to that observed for D-serine, and this effect was not due to direct increases in endogenous D-serine.

Acknowledgment We thank Meiji Pharmaceutical for providing 2 g of D-cycloserine. Funding for this study was partly provided by a grant from the Minister of Education, Culture, Sports, Science, and Technology of Japan (to E.S. and K.H.).

Conflict of interest statement The authors declare no conflict of interest except for 2 g of D-cycloserine transferred from Meiji Pharmaceutical.

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