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Short communication

Differential effects of glycine on the anticonvulsant activity of D-cycloserine and L-701,324 in mice

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Abstract:
The anticonvulsant effects of D-cycloserine, which is a partial agonist of the glycine/*N*-methyl-D-aspartate (NMDA) receptor, and L-701,324, which is a selective and potent antagonist that acts at the glycine site, were studied in electroshock-induced seizures in mice. Glycine, which is a natural full agonist that acts at the glycine site, enhanced the seizure threshold-increasing effect of D-cycloserine. L-701,324 produced a marked increase in the seizure threshold, which was significantly reversed by the administration of glycine. These results suggest that indirect glycine/NMDA antagonistic mechanisms may be responsible for the anticonvulsant action of D-cycloserine.

Key words:
D-cycloserine, glycine, L-701,324, NMDA, maximal electroshock, seizure threshold, mice

Abbreviations: ANOVA – analysis of variance, CC_{50} – electric current in mA necessary to produce a tonic extension of the hind limbs in 50% of the stimulated mice, MEST – maximal electroshock seizure threshold test, NMDA – *N*-methyl-D-aspartate

Introduction

D-Cycloserine is a moderate efficacy partial agonist that acts at the strychnine-insensitive glycine modulatory site within the *N*-methyl-D-aspartate (NMDA)-receptor/ionophore complex [8]. The anticonvulsant effects of D-cycloserine were reported in several models of epilepsy, including maximal electroshock seizures and pentylenetetrazole-induced tonic seizures, and in the kindling model of epilepsy [10–12, 17, 18]. The mechanism of the anticonvulsant action of D-cycloserine is not fully delineated. Because its intrinsic efficacy at the glycine site represents 40–70% of the intrinsic efficacy of glycine [8], the anticonvulsant effects of D-cycloserine might be attributed to either an agonist (desensitization of the NMDA receptor) or antagonist effect (inhibition of the NMDA receptor). Therefore, we investigated the anticonvulsant activity of D-cycloserine in the absence or presence of exogenous glycine, which is

a natural full agonist at the glycine site. The potent antagonist L-701,324 that acts at this site and has adequate central availability after systemic administration [3, 7, 13, 14, 19] was used for comparison with D-cycloserine.

Materials and Methods

The experiments were performed on experimentally naive male Swiss mice weighing 23–27 g and maintained under standard environmental conditions. The experimental protocol was approved by the institutional ethical committee, and all the procedures complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

The maximal electroshock seizure threshold (MEST) test, which is a sensitive and reliable test to determine the threshold of grand-mal type seizures in humans [9], was used in mice. Convulsive current (CC_{50}) values, which were defined as the electric currents (in mA) that were necessary to produce a tonic extension of the hind limbs in 50% of mice subjected to the procedure, were determined using an 'up-and-down' procedure [6, 15]. Groups of 20 mice were injected with drugs or vehicles at the indicated time points and were subsequently stimulated transcorneally (50 Hz, 0.2 s). A tonic extension of the hind limbs was taken as the endpoint. The intensity of an electric stimulus that was generated by a constant current stimulator decreased or increased by 0.06-log intervals depending on whether the previously stimulated animal did or did not exhibit the endpoint, respectively. This sensitive method allowed precise determination of CC_{50} values and confidence limits for 95% probability in small groups of mice because the stimulation level was maintained near the actual CC_{50} value.

D-cycloserine (D-4-amino-3-isoxazolidone, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in distilled water and injected subcutaneously (*sc*) at a dose of 320 mg/kg 60 min before testing. Glycine (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in water and injected intraperitoneally (*ip*) at a dose of 200 mg/kg 4 h before electroconvulsions. L-701,324 (7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2(*H*)quinolone, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in water that was slightly alkalinized with NaOH and administered *sc* at a dose of 2 mg/kg 30 min prior to the test. Glycine was initially tested alone and found to be ineffective at the dose used in this study. The control mice were injected with the appropriate vehicles. All test solutions were injected at a volume of 10 ml/kg. The doses of the drugs and pretreatment times were selected based on a literature search and were confirmed during preliminary experiments.

The data were analyzed using the one-way analysis of variance (ANOVA) with Student-Newman-Keuls *post-hoc* test. A p-value less than or 0.05 was considered significant.

Results and Discussion

Administration of D-cycloserine at a dose of 320 mg/kg resulted in a significant threshold increase by 66%. Administration of glycine alone (200 mg/kg, *ip*) induced no anticonvulsant effect or neurotoxicity. Glycine at a dose of 200 mg/kg was administered 4 h before the test and significantly potentiated the ability of D-cycloserine to increase the seizure threshold (Fig. 1A). Similar to D-cycloserine, L-701,324 was administered at a dose of 2 mg/kg and increased the seizure threshold by approximately 90%. Pretreatment of the animals with glycine at a dose of 200 mg/kg significantly reduced the antielectroshock action of L-701,324 (Fig. 1B).

The antagonists that act at the glycine site within the NMDA-ionophore complex, such as L-701,324, possess anticonvulsant properties that correlate with a reduction of the glutamatergic excitatory input [1]. However, the anticonvulsant effects of D-cycloserine are somewhat unexpected because agents with an agonist activity at the glycine site allosterically amplify glutamate responses of the NMDA-ionophore complex. We demonstrated that glycine reversed the antagonist-induced increase but not the D-cycloserine-induced increase in the seizure threshold. In contrast to L-701,324, the anticonvulsant effects of D-cycloserine cannot be explained by glycine antagonism. These results are in agreement with those of other reports showing that the anticonvulsant effects of D-cycloserine are antagonized by an antagonist [11] and a low-efficacy partial agonist at the glycine site [10].

Glycine is an absolute requirement for NMDA receptor activation and controls NMDA receptor functional state [2, 4]. A previous study has shown that

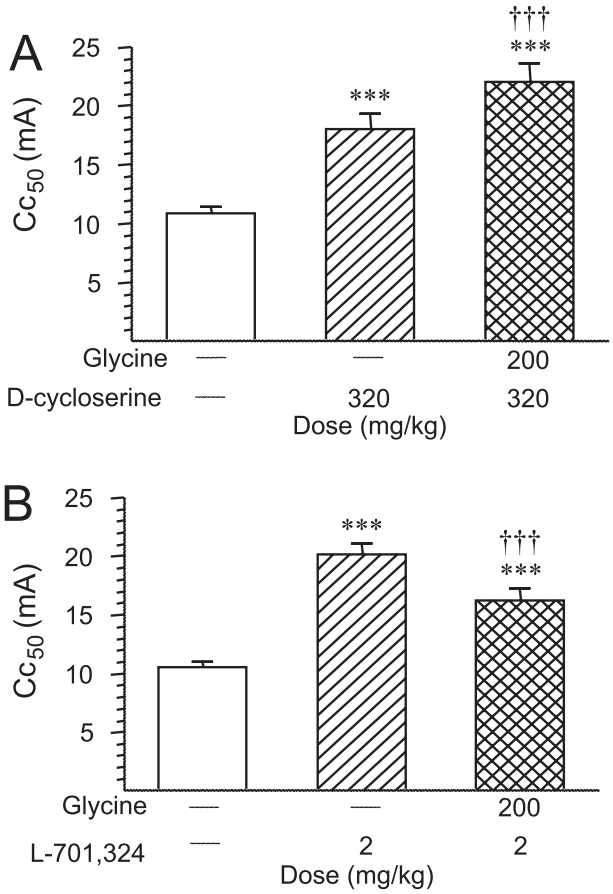

Fig. 1. Differential effects of glycine on the anticonvulsant activity of D-cycloserine and L-701,324 in mice. The seizure threshold was determined using an 'up-and-down' method (for details, see Materials and Methods). The data are shown as seizure threshold values in mA with upper confidence limits for 95% probability. Glycine, D-cycloserine and L-701,324 were administered *ip* 4 h, *sc* 60 min and *sc* 30 min, respectively, before the test. Administration of glycine alone at a dose of 200 mg/kg did not induce anticonvulsant effects or neurotoxicity. D-cycloserine (panel **A**): ANOVA $F(2, 25) = 142.91$, $p < 0.0001$; *** $p < 0.001$ *vs.* the control group (glycine and D-cycloserine-untreated animals), ††† $p < 0.001$ *vs.* the D-cycloserine-treated mice (Student-Newman-Keuls *post-hoc* test). L-701,324 (panel **B**): ANOVA $F(2, 26) = 138.69$, $p < 0.0001$; *** $p < 0.001$ *vs.* the control group (animals that were not treated with glycine and L-701,324), ††† $p < 0.001$ *vs.* L-701,324-treated mice (Student-Newman-Keuls *post-hoc* test)

chronic but not acute treatment with another high-efficacy partial agonist, 1-aminocyclopropanecarboxylic acid (ACPC), at the glycine site reduces forebrain ischemia in gerbils [16]. Therefore, it is reasonable to speculate that an acute high dose of a high-efficacy partial agonist may desensitize the NMDA receptor yielding pharmacological effects that are similar to those of 'typical' competitive and noncompetitive NMDA antagonists. We hypothesized that in specific conditions, high efficacy partial agonists at the glycine site may functionally antagonize the NMDA receptor. This hypothesis is supported by the findings that D-cycloserine and a full agonist, D-serine, but not a low efficacy partial agonist, *R*-(+)-HA-966, at the glycine site, increases the afterdischarge threshold in a model of focal epilepsy in rats [10]. In addition, glycine transporter type 1 inhibitors may also have anticonvulsant properties [5, 15]. It is noteworthy that the changes following D-cycloserine administration last for several days [10]. Because D-cycloserine has a short half-life, these changes may indicate the adaptive effects on the NMDA receptor complex in response to the glycine site stimulation rather than to the direct antagonism at the receptor level. Although this study does not elucidate the mechanism of anticonvulsant action of D-cycloserine, it shows that possibly indirect glycine/NMDA antagonistic mechanisms may also be involved.

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