0031-3998/08/6403-0228
PEDIATRIC RESEARCH

Vol. 64, No. 3, 2008
Printed in U.S.A.

ARTICLES

Model Mice for Mild-Form Glycine Encephalopathy: Behavioral and Biochemical Characterizations and Efficacy of Antagonists for the Glycine Binding Site of N-Methyl D-Aspartate Receptor

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ABSTRACT: Glycine encephalopathy (GE) is caused by an inherited deficiency of the glycine cleavage system (GCS) and characterized by accumulation of glycine in body fluids and various neurologic symptoms. Coma and convulsions develop in neonates in typical GE while psychomotor retardation and behavioral abnormalities in infancy and childhood are observed in mild GE. Recently, we have established a transgenic mouse line (low-GCS) with reduced GCS activity (29% of wild-type (WT) C57BL/6) and accumulation of glycine in the brain (Stroke, 2007; 38:2157). The purpose of the present study is to characterize behavioral features of the low-GCS mouse as a model of mild GE. Two other transgenic mouse lines were also analyzed: high-GCS mice with elevated GCS activity and low-GCS-2 mice with reduced GCS activity. As compared with controls, low-GCS mice manifested increased seizure susceptibility, aggressiveness and anxiety-like activity, which resembled abnormal behaviors reported in mild GE, whereas high-GCS mice were less sensitive to seizures, hypoactive and less anxious. Antagonists for the glycine-binding site of the N-methyl-D-aspartate receptor significantly ameliorated elevated locomotor activity and seizure susceptibility in the low-GCS mice. Our results suggest the usefulness of low-GCS mice as a mouse model for mild GE and a novel therapeutic strategy. **(*Pediatr Res* 64: 228–233, 2008)**

Glycine encephalopathy (GE) is an inborn error of metabolism characterized by glycine accumulation in plasma, cerebrospinal fluid and various organs including the brain (1). The fundamental defect of GE lies in the mitochondrial glycine cleavage system (GCS). The GCS is an enzyme complex, which breaks down glycine to carbon dioxide, ammonia and one-carbon units. The GCS is composed of four individual proteins: glycine decarboxylase (GLDC), aminomethyl transferase, aminomethyl carrier protein, and lipoamide dehydrogenase, which are encoded by *GLDC*, *AMT*, *GCSH* and *GCSL*, respectively (2). A defect of any component can lead to defective overall activity of the GCS. *GLDC*, *AMT* and *GCSH* mutations have been reported in GE patients (3,4). Patients with typical GE have severe symptoms such as convulsions, respiratory failure and lethargy in the neonatal period, frequently leading to death. Patients with mild GE do not experience coma or convulsive seizures in the neonatal period, but manifest developmental delay and behavioral abnormalities in infancy and childhood (5–7). They tend to be hyperirritable, hyperactive and aggressive characteristics, which are often problematic for their families. Patients with mild GE are thought to have increased susceptibility to seizures. Mild GE tends to be characterized by higher GCS residual activity than typical GE (8,9). No effective therapy has been established for either typical or mild GE.

Glycine is a major neurotransmitter, which plays a dual role in CNS. It acts on the inhibitory glycine receptor in spinal cord and brainstem (10), as well as a coagonist of N-methyl-D-aspartate-sensitive (NMDA) glutamate receptors in the cortex, hippocampus, cerebellum and brainstem (11). Enzymatic activity of the GCS is high in the forebrain and cerebellum and low in the spinal cord, while the inhibitory glycine receptors are mainly distributed in the brainstem and spinal cord, suggesting that the GCS is co-localized with NMDA receptors (12,13). Therefore, the GCS would play a role in regulation of extracellular glycine concentration in the vicinity of NMDA receptors, rather than the inhibitory glycine receptors.

Recently, we generated a transgenic mouse line, designated as low-GCS, expressing dominant-negative *GLDC* cDNA to reduce GCS activity (14). The low-GCS mouse has 29% of normal GCS activity in CNS, and shows elevated glycine concentration in extracellular fluid. A transgenic mouse line over expressing normal glycine decarboxylase cDNA, desig-

Received November 6, 2007; accepted April 3, 2008.
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Financial support: This work was supported by a grant from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and a grant from the Ministry of Health, Labour, and Public Welfare in Japan.

Abbreviations: GCS, glycine cleavage system; **GE,** glycine encephalopathy; **GLDC,** glycine decarboxylase; **NMDA,** N-methyl-D-aspartate; **5-HT**, serotonin

nated as high-GCS, was also established, which showed elevated GCS activity and a lower extracellular concentration of glycine in CNS. Using these two mouse lines, we have found that ischemic injury depends on the extracellular level of glycine. We have also demonstrated that an antagonist for the glycine site of the NMDA receptor significantly ameliorates the ischemic injury in low-GCS mice.

The purpose of this study is to characterize the behavioral features of the low-GCS mouse as a model for mild GE by examining locomotor activity, seizure susceptibility, aggressiveness and anxiety-related behavior, which are reported as behavioral features associated with patients with mild GE. We compared the low-GCS mice not only with wild-type (WT) C57BL/6 mice, but also with high-GCS mice and another transgenic mouse line with low GCS activity designated as the low-GCS-2 mouse line. Results of an experimental therapy in which antagonists of the NMDA glutamate receptor were applied to those model mice suggest the possibility of a novel treatment of mild GE.

MATERIALS AND METHODS

This study was performed according to fundamental guidelines of proper conduct of animal experiment and related activities in Academic Research Institutions of Japan. Animal experiment committee of Tohoku University School of Medicine approved this study (approval number, 8-26, 1998).

Transgenic mice. Establishment and characterization of two transgenic mouse lines, high-GCS and low-GCS, have been previously reported (14). In this study we used another mouse line with transgenic expression of a dominant-negative GLDC, designated as low-GCS-2, which was established by injection of the same transgenic vector with the low-GCS mouse (Fig. 1*A*). The low-GCS-2 mice showed accumulation of glycine in the cortex homogenate and extracellular fluids in the striatum (Table 1). Both low-GCS and low-GCS-2 mice manifested no brain malformations such as hypogenesis of corpus callosum, which are frequently observed in patients with typical GE. Experimental mice were kept in a controlled environment at 21 ± 2°C and 40–60% air humidity in a 12/12 h light/dark cycle with food and water *ad libitum*. C57BL/6 mice were purchased from Japan SLC Co. Ltd. (Hamamatsu, Japan). Each transgenic mouse line was backcrossed with C57BL/6 mice more than 10 generations and used for behavioral studies.

Measurement of locomotor activity. The locomotor activity of the mice was measured by sensor, which detected infrared energy emitted by the mouse (Supermex; Muromachi Kikai, Tokyo, Japan). The mice were placed in a novel test cage (30 × 20 × 13 cm) with paper-chip bedding, and measurement was commenced 30 min later. Spontaneous locomotor activity of 6 to 8 wk-old female mice was measured for 24 h. Data were expressed as mean ± SD and analyzed by one-way analysis of variance (ANOVA) with the *post hoc* Fisher's PLSD test.

Seizure induced by an electroshock. Seizure susceptibility was evaluated by measuring the durations of each phase of convulsion induced by electroshock (15). The tonic phase was regarded as the period between the onset of hind-limb extension and the beginning of myoclonic jerks, the clonic phase as that during myoclonic jerks and the convulsive coma phase as that between the end of myoclonic jerks and the recovery of the righting reflex. Data were expressed as mean ± SD and analyzed by one-way ANOVA with the *post hoc* Fisher's PLSD test.

Resident-intruder test. A resident-intruder test has been developed for evaluation of male aggressiveness (16). Male mice, 12–18 wk of age (residents) were kept isolated in separate cages for at least 2 wk. The home cages of the resident mice were not changed during isolation. A group-housed Balb/c male mouse was used as an intruder. Residents were previously exposed to an intruder twice a week for a total of four 15-min confrontations. When an intruder was introduced into the home cage of the resident at the fifth confrontation, the latency to the first attack bite was noted and the total number of attack bites was recorded for 15 min. A mouse that had had no previous contact with the resident was used as the intruder at each confrontation. We defined latency less than 30s as short latency and more than 30 attacks as frequent attack. The proportion of short latency and frequent attacks in each mouse line was compared with that of the WT mice by Fisher's exact test.

Elevated-plus maze. The elevated-plus maze test is widely used for evaluation of anxiety in rodents (17). The plus maze apparatus was purchased from Muromachi Kikai, Co., Ltd., was made of black Plexiglas and consisted of two open arms (30 × 5 cm) and two closed arms (30 × 5 cm). The four arms extended from a central platform (5 × 5 cm). After adaptation to laboratory conditions for two days, a mouse was placed on the central

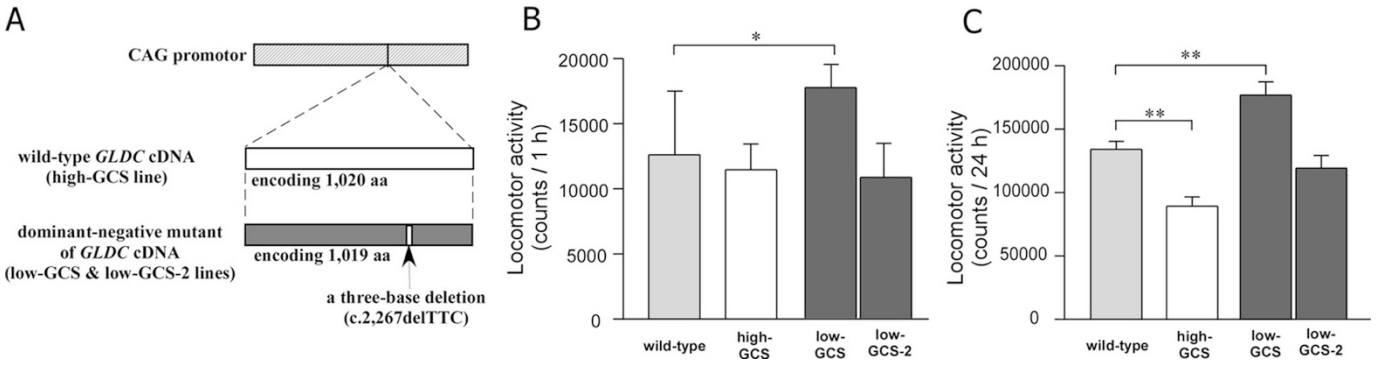

Figure 1. Locomotor activity. (*A*) Structure of the transgenes. WT and dominant-negative mutant of human *GLDC* cDNA were inserted under the CAG promoter for generation of transgenic mice. High-GCS mouse line expressed WT GLDC enzyme and had higher GCS activity. Low-GCS and low-GCS-2 mouse lines expressed the dominant-negative GLDC enzyme and had lower GCS activity. (*B*, *C*) Locomotor activity of mutant mice with altered GCS activity. Total locomotor activity was measured for 1 h (*B*) and 24 h (*C*). Low-GCS mice manifested significantly higher locomotor activity than did WT mice in both experiments of 1 h and 24 h. High-GCS mice showed a significantly lower locomotor activity in the 24 h experiment. *Column*, means of 8–9 mice; *bar,* S.D. $*p < 0.05$; $**p < 0.01$ (*vs*. WT mice).

Table 1. *Cerebral glycine concentrations in the transgenic mouse lines*

	Wild-type§	High-GCS§	Low-GCS§	Low-GCS-2
Brain homogenates (μmol/gram tissue)				
Cortex	0.90 ± 0.05	0.71 ± 0.06*	1.36 ± 0.06**	1.08 ± 0.1*
Striatum	1.00 ± 0.03	0.94 ± 0.04	1.22 ± 0.05*	1.08 ± 0.03
Extracellular concentration (μmol/L)				
Striatum	1.0 ± 0.1	0.6 ± 0.1**	1.4 ± 0.1**	1.2 ± 0.1**

* $P < 0.05$; ** $P < 0.01$ (vs. the wild-type mice). § reported previously (14).

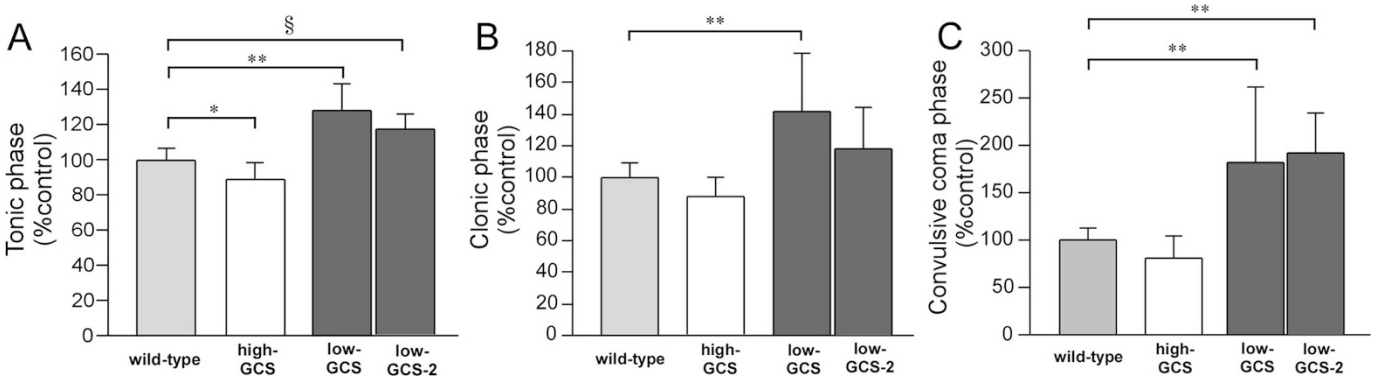

Figure 2. Seizure susceptibility. (*A–C*) Duration of each seizure phase is expressed as % of the mean of the WT mice. The durations of the tonic, clonic and convulsive-coma phases were significantly longer in the low-GCS mice as compared with the WT mice. *Column*, means of 6–12 mice; *bar*, S.D. $*p < 0.05$; $**p < 0.001$; §$p < 0.0001$ (*vs*. WT mice).

platform facing an open arm. The number of entries into the open and closed arms and the time spent in each arm were measured for 5 min. A mouse was considered to have entered an arm when all four legs were in the arm. When a mouse spent less time in an open arm, it was considered to be more anxious. Data were expressed as mean ± SD and analyzed by one-way ANOVA with the *post hoc* Fisher's PLSD test.

Drugs. L-701,324, (+)-HA-966 and MK-801 were obtained from Tocris Cookson (UK). L-701,324 is an antagonist for glycine binding site of NMDA receptor. (+)-HA-966 is a partial agonist of glycine binding site of NMDA receptor. MK-801 is a blocker of NMDA receptor channel. The antagonists were intraperitoneally injected 30 min before starting the test. Then 12-h locomotor activity at nocturnal period of mice was measured using Supermex as described above. L-701,324 (1.25, 2.5, 5 mg/kg), (+)-HA-966 (3, 10, 30 mg/kg) and MK-801 (0.1, 0.5, 1.0 mg/kg) were administered to WT and low-GCS mice (3–6 mo old, $n = 7–12$). The locomotion of each mouse was measured in each mouse after saline injection (10 mL/kg of body weight) and was used as the baseline value of locomotion ($n = 13–16$). Data were expressed as mean ± SE, which were analyzed by unpaired *t* test.

Analysis of monoamines and their major metabolites in CNS. The amount of monoamines and their metabolites was determined by reverse-phase HPLC with electrochemical detection as described by Miura *et al.* (18). Mice were killed by deep diethylethel anesthesia of for isolation of brains, which were dissected into the cerebral cortex and hippocampus, and stored at −80°C until analysis. Data were expressed as mean ± SE and analyzed by one-way ANOVA with the *post hoc* Fisher's PLSD test. P-values were subjected to Bonferroni's correction (multiplied by five).

RESULTS

Biochemical characterization of low-GCS-2 mouse line. Glycine concentrations in brain homogenate and extracellular fluids of the low-GCS-2 mice are summarized in Table 1. Extracellular glycine concentration measured in the striatum by microdialysis method was significantly high in low-GCS-2 mice as compared with the WT C57BL/6 mice. Glycine concentration was significantly elevated in cortex homogenate, but not in striatum homogenate. The whole brain GCS activity of low-GCS-2 mice was 33% of that of WT C57BL/6 mice determined by decarboxylation assay with [1-^{14}C]glycine as described (19).

Locomotor activity. Twenty-four-hour monitoring showed that spontaneous locomotor activity was significantly higher in low-GCS mice than in the WT mice. In contrast, the activity was significantly lower in the high-GCS mice than in the WT mice (Fig. 1C).

Seizure susceptibility. The duration of tonic seizure induced by electric shock was significantly shorter in high-GCS mice than WT mice (Fig. 2). In contrast, both low-GCS and low-GCS-2 mice had significantly longer tonic phase than WT mice. The clonic phase had significantly longer duration in low-GCS mice. The duration of convulsive-coma phase was significantly longer in both low-GCS and low-GCS-2 mice as compared with WT mice.

Aggressiveness. In the resident-intruder test, 8.3%, 25%, 53.8% and 54.3% of the mice started attacking within 30 s after initiation of the test in the WT, high-GCS, low-GCS and low-GCS-2 mice, respectively (Fig. 3*A–D*), ratios of these earlier attackers being significantly higher in the low-GCS and low-GCS-2 mice than in the WT mice. The number of attack bites in 15 min was significantly higher in the low-GCS (43.0 ± 8.5) and low-GCS-2 mice (44.0 ± 6.7) than in the WT mice (17.0 ± 2.8) (Fig. 3*E*), suggesting elevated aggressiveness in the low-GCS and low-GCS-2 mice.

Anxiety-like behavior. In the elevated-plus maze test, the two mouse lines with reduced GCS activity, low-GCS and low-GCS-2 mice, spent significantly less time in the open arms than did the WT mice (Fig. 3*C*). In contrast, the high-GCS mice spent a significantly longer time in the open arms than did the WT mice. This result suggests a correlation between extracellular glycine concentration and anxiety-related behavior.

Effect of antagonists for NMDA receptor on locomotor activity and seizure susceptibility. Glycine antagonists with different binding sites for the NMDA receptor were administered to the wild type mice and low-GCS mice and their locomotor activities were measured. When saline was administrated, the low-GCS mice showed greater hyperactivity throughout the dark phase as compared with the WT mice (Fig. 4*A*). Antagonists of the glycine-site of NMDA receptor, L-701,324 and (+)HA-966, decreased nocturnal locomotor activity in the low-GCS mice and locomotor pattern in the WT mice did not apparently change (Fig. 4*B*, *C*). An antagonist of the whole NMDA receptor channel, MK-801, increased locomotor activity in both the WT and low-GCS mice for several hours after administration, the low-GCS tended more active than wild in nocturnal period (Fig. 4*D*). L-701,324, a full antagonist of the glycine site of the NMDA receptor, significantly lowered the locomotor activity of the low-GCS mice to a level similar to that of the WT mice at a dose of 1.25 mg/kg or more, whereas it did not have a significant effect on the wild- type mice up to a dose of 5.0 mg/kg (Fig. 4*E*). Similarly,

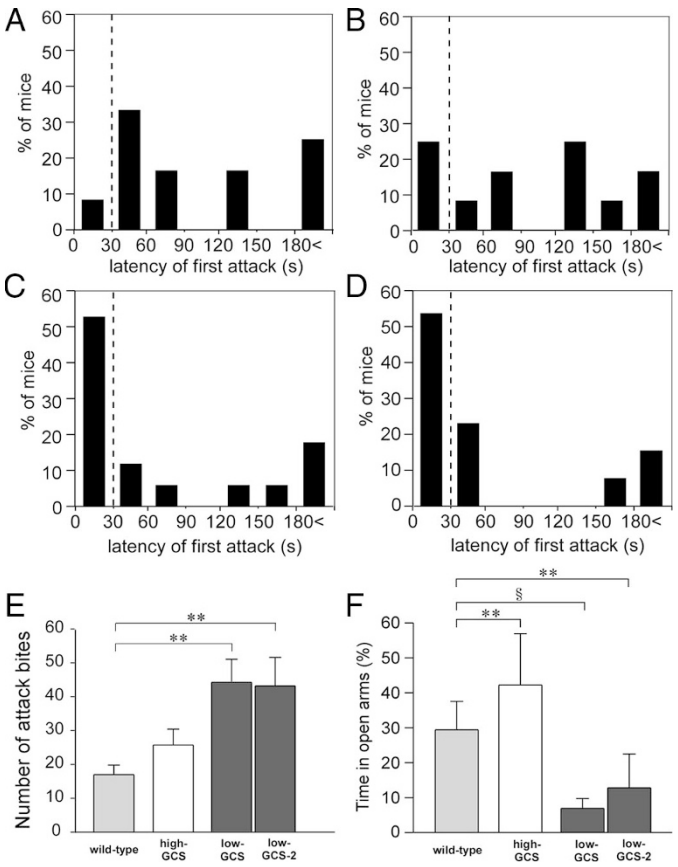

Figure 3. Aggressive and anxiety-like behaviors in transgenic mice. (*A–D*) The latency of the first attack in the resident-intruder test in WT (*A*), high-GCS (*B*), low-GCS (*C*) and low-GCS-2 mice (*D*). The percentage of the total tested mice (n = 12–17 in each group) is shown. The low-GCS and low-GCS-2 mice had shorter latency of the first attack compared with the WT and high-GCS mice. (*E*) Number of attack bites in the resident-intruder test. The results are shown as mean ± SE (n = 12–17 in each group). (*F*) The elevated-plus maze test. The percentage of the time spent in open arms during the test time is shown. *Column*, means of 7–10 mice; *bar*, S.D. $^{**}p < 0.01$; $\S p < 0.0001$ (*vs*. WT mice).

(+)-HA966, a partial agonist of the glycine site of the NMDA receptor, reduced locomotor activity only in low-GCS mice of a dose of 5–30 mg/ kg (Fig. 4*F*). MK-801 showed no effect at any dose in WT or low-GCS mice (Fig. 4*G*). The effect of L-701,324 on seizure susceptibility was evaluated by the same method as in Fig. 2. The duration was significantly ($p < 0.05$) shorter with administration of 2.5 mg/kg of L-701,324 than with saline (Fig. 4*H*). L-701,324, however, did not significantly reduce the duration of the clonic phase or the convulsive and coma phase.

Monoamine metabolism. Monoamine concentrations of the three mouse lines were measured using homogenates of cortex and hippocampus (Table 2). The high-GCS had significantly decreased levels of NE and 5-HT, and increased 5-HIAA/ 5-HT ratio in the cortex. No significant difference was observed in other monoamine levels.

DISCUSSION

We studied biochemical and behavioral features of three transgenic mouse lines with distinct GCS activity. The low-GCS and low-GCS-2 mouse lines had reduced GCS activity and higher extracellular glycine concentrations, while the high-GCS mice had elevated GCS activity and lower extracellular glycine concentration. The behavioral features of the low-GCS mice were compared with those associated with mild GE (5–7). Patients with mild GE have hyperactivity, increased sensitivity to seizures, hyperirritability and temper tantrums, which are sometimes followed by outbursts of aggressiveness and rage. Patients with mild GE are sometimes given a diagnosis of attention deficit hyperactive disorder resistant to treatment with psychostimulants. In contrast to classical GE, in which intractable seizures are commonly observed, some patients with mild GE manifest febrile convulsions rather than epileptic seizures. In this study, the low-GCS mice showed the behavioral abnormalities such as elevated seizure susceptibility, locomotor activity, aggressiveness and anxiety, which resemble those associated with mild GE, suggesting that the low-GCS mouse line may possibly be a useful mouse model for mild GE.

In the several behavioral tests, the high-GCS mice with glycine depletion showed features opposite those manifested by the low-GCS mice with glycine accumulation. The former showed less locomotor and anxiety-like activity than the WT mice. We found the low-GCS mice to be more vulnerable to ischemic injury than the high-GCS mice in the previous study (14). Extracellular glycine concentration may be, therefore, a major determinant of susceptibility to seizure, ischemic vulnerability, locomotor activity and mood. The amelioration of behavioral abnormalities, hypersensitivity to seizures and ischemic injury by antagonists for the glycine-binding site of NMDA receptor in low-GCS mice suggests that those abnormal features may be caused by the overexcitation of the NMDA receptor. Extracellular glycine level can be elevated by gene knockout of a glycine transporter, GlyT1 (20). The homozygous mice died within 12 h of birth. Locomotor activity of heterozygous mice for GlyT1 gene knockout was comparable with the WT mice, suggesting that the partial deficiencies of both GlyT1 and GCS in mice cause high extracellular glycine, but different effect on locomotor activity. Glycine administration to normal humans and animals has been shown not to cause any behavioral change, which is considered to be due to poor penetration of the blood brain barrier by glycine (21). Administration of high-dose glycine, however, has been found to ameliorate negative symptoms of schizophrenia in affected individuals and model animals for schizophrenia, which is considered to be based on the NMDA receptor hypofunction theory of schizophrenia (22). A slight elevation of glycine may have an effect in specific pathologic conditions such as schizophrenia or in certain individuals sensitive to glycine.

The results of the present behavioral studies of low-GCS mice agree with the previous reports of local administration studies of glycine and its antagonists, in which microinjection of glycine into dorsal periaqueductal gray matter caused anxiety-like activity and antagonits of the NMDA glycine site reduced anxiety in rodents (23). In rats, extracellular microperfusion of glycine in the hippocampus have been lower the picrotoxin-induced seizure threshold (24). There are sev-

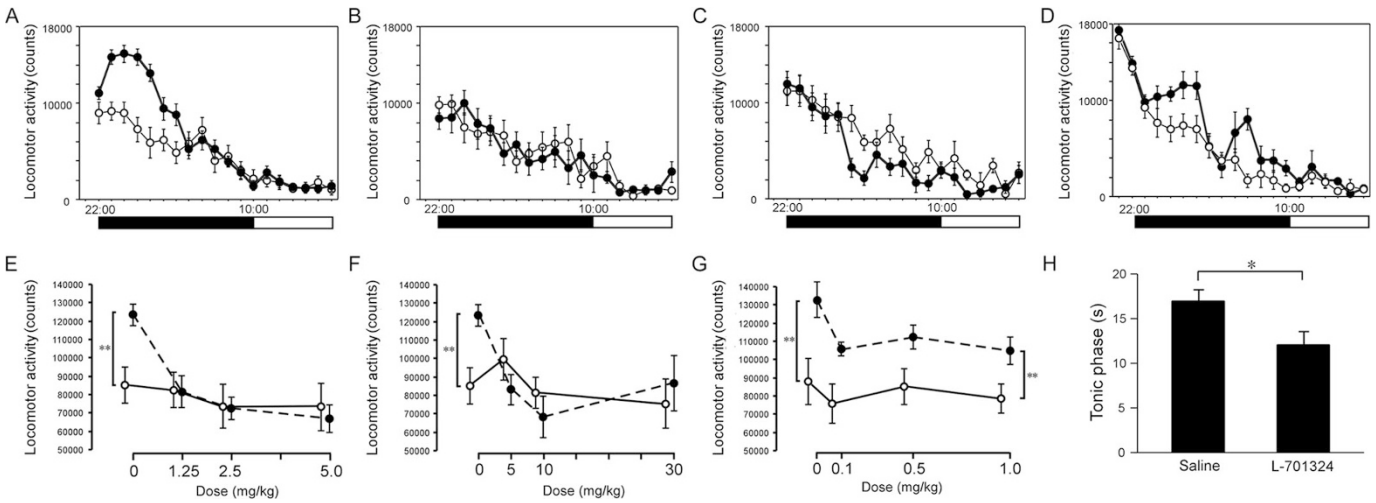

Figure 4. Effect of antagonists of NMDA receptor. (*A–D*) The locomotor activity over an 18-h period was measured in WT mice (open circles) and low-GCS mice (closed circles) one hour after peritoneal injection of following solutions: saline (*A*), 1.25 mg/kg of L-701,324 (*B*), 5 mg/kg of (+)-HA-966 (*C*) and 0.1 mg/kg of MK-801 (*D*). *Dot*, mean, *bar,* S.E. ($n = 6–12$). Black bar below the *x* axis represents dark phase (22:00 ~ 10:00), and white bar represents light phase (10:00 ~ 6:00). (*E–G*) The total activity of the nocturnal locomotor (12 h) of the low-GCS mice (closed circles) was compared in the WT mice (open circles) after various doses of administration of L-701,324 (*E*), (+)-HA-966 (*F*) or MK-801 (*G*). (*H*) Effect of L-701324 on seizure susceptibility. Duration of tonic phase was measured in electric shock-induced convulsion in low-GCS mice. One hour before the test, 2.5 mg/kg of L-701,324 was intraperitoneally administered. *Column*, means of 8–11 mice; *bar*, S.D. $*p < 0.05$; $**p < 0.01$ (*vs*. WT mice).

Table 2. *Cerebral monoamine concentrations in the transgenic mouse lines*

	Mouse line			
	Wild-type	High-GCS	Low-GCS	Low-GCS-2
Cortex				
DA	331 ± 241	348 ± 232	1181 ± 752	291 ± 196
DOPAC	55 ± 33	68 ± 43	150 ± 54	70 ± 44
NE	654 ± 88	538 ± 41*	565 ± 34	616 ± 79
5-HT	914 ± 92	731 ± 74**	798 ± 51	861 ± 107
5-HIAA	408 ± 88	420 ± 73	334 ± 31	438 ± 66
DOPAC/DA	0.192 ± 0.040	0.230 ± 0.080	0.151 ± 0.058	0.166 ± 0.057
5-HIAA/5-HT	0.445 ± 0.068	0.575 ± 0.078*	0.420 ± 0.041	0.515 ± 0.104
Hippocampus				
DA	334 ± 205	643 ± 466	261 ± 80	461 ± 357
DOPAC	28 ± 39	117 ± 84	50 ± 8	118 ± 97
NE	464 ± 79	484 ± 85	434 ± 94	441 ± 84
5-HT	886 ± 153	868 ± 83	841 ± 230	842 ± 159
5-HIAA	689 ± 105	745 ± 198	553 ± 140	815 ± 133
DOPAC/DA	0.194 ± 0.094	0.207 ± 0.046	0.204 ± 0.055	0.194 ± 0.094
5-HIAA/5-HT	0.788 ± 0.114	0.868 ± 0.256	0.676 ± 0.172	0.976 ± 0.100

Levels of DA, DOPAC, NE, 5-HT and 5-HIAA in the cerebral cortex, striatum and hippocampus in each strain were determined. Values are expressed in nanograms per gram wet tissue. The results are shown as mean ± S.D. ($n = 7–8$ in each group). The data were analyzed by one-way of variance (ANOVA) and the *P*-values were subjected to Bonferroni's correction (multiplied by 5). * $p < 0.05$; ** $p < 0.01$ (vs wild-type mice).

NE, norepinephrine; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid.

eral subtypes of inhibitory glycine receptors (25) and NMDA receptors (26). These receptor subtypes are expressed in different CNS regions and have distinct reactivity, causing specific behavioral change when the glycine level in a specific region is altered. Chronic glycine treatment has been found to cause the transient hypertrophy of astrocytes and reduction of Ca^{2+} channels (27). It has also been reported that desensitization to 1-aminocyclopropanecarboxylic acid after chronic treatment of glycine was not a direct effect of glycine but occurred due to adaptation of the NMDA receptor (28). Adaptation to a high concentration of extracellular glycine may affect the behavior observed in low-GCS mice.

One possible explanation for the behavioral changes in low-GCS and/or high-GCS mice is that abnormal glycine metabolism alters the metabolism of neurotransmitters, which play a pivotal role in the control of behavior. Glycine has been shown to affect the neural release of noradrenalin (29) and release of dopamine *in vivo* (30). These studies prompted us to measure concentrations of monoamines and their metabolites in the four mouse strains (Table 2). Several significant changes were indeed detected in the high-GCS mice. Those changes of monoamine concentrations, however, appeared complex, and could not directly explain the behavioral abnormalities. There was not any common change of monoamine concentration in the low-GCS and low-GCS-2 mice, nor any opposite-direction changes in low-GCS *versus* high-GCS mice, suggesting that monoaminaergic changes may contribute behavioral changes in a more complex manner than we

initially expected. Abnormal metabolism of other amino acid neurotransmitters such as GABA and D-serine is another possible explanation for the behavioral abnormality. A transgenic mouse expressing a mutant subunit of the inhibitory glycine receptor was found to show drastically reduced $GABA_A$-receptor-mediated transmission (31). D-serine is another ligand of the NMDA glycine site and has been observed to be elevated in the brain of GE patients (32). Analysis of other neurotransmitters is required for further understanding of the pathogenesis of behavioral abnormalities.

Many therapeutic approaches for the treatment of classical GE have been suggested (1). To facilitate glycine excretion into urine sodium benzoate is used, which is effective for reduction of the glycine level in plasma, but is of limited effect for the reduction of the glycine level in cerebrospinal fluids. Antagonists such as strychnine for inhibitory glycine receptor have been also used, but their efficacy has not been established and they are presently rarely used. The administration of several antagonists for the NMDA channel has been attempted. Dextromethorphan has improved EEG findings and consciousness level although its efficacy on log-term prognosis has not been established (1). Two other reports have discussed treatment of abnormal behaviors associated with mild GE. One report described the efficacy of imipramine (33), which is an anti-depressant and a weak antagonist for the NMDA receptor. In the cited report, a combination therapy of imipramine and sodium benzoate was found to ameliorate developmental scores. The other report describes the efficacy of typtophan (34). In that report, treatment with tryptophan was found to improve the IQ of a patient with mild GE. Tryptophane is metabolized into kynureine, an antagonist for the glycine-binding site of the NMDA receptor.

In the present study, we demonstrated the existence of two antagonists for the glycine-binding site of the NMDA receptor. L-701,324 and (+)-HA-966, were found to be effective for treatment of the abnormal behaviors observed in low-GCS mice. To date, a number of antagonists for the glycine-binding site of the NMDA receptor have been developed for the purpose of treatment of stroke, and the clinical safety of them has been confirmed. Based on our study of model mice, we would like to propose the use of antagonists for the glycine-binding site of the NMDA receptor for treatment of GE, especially for treatment of behavioral abnormalities associated with mild GE. The model mice used in the present study would be useful for evaluation of the efficacy of such antagonists in the treatment of GE.

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