Full Paper

Neuroprotection via Strychnine-Sensitive Glycine Receptors During Post-ischemic Recovery of Excitatory Synaptic Transmission in the Hippocampus

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Abstract. Recent evidence indicates that strychnine-sensitive glycine receptors are located in upper brain regions including the hippocampus. Because of excitatory effects of glycine via facilitation of NMDA-receptor function, however, the net effects of increased extracellular glycine on neuronal excitability in either physiological or pathophysiological conditions are mostly unclear. Here, we addressed the potential neuroprotective effect of either exogenous application of glycine and taurine, which are both strychnine-sensitive glycine-receptor agonists, or an endogenous increase of glycine via blockade of glycine transporter 1 (GlyT1) by assessing their ability to facilitate the functional recovery of field excitatory postsynaptic potentials (fEPSPs) after termination of brief oxygen/glucose deprivation (OGD) in the CA1 region in mouse hippocampal slices. Glycine and taurine promoted restoration of the fEPSPs after reperfusion, but this was never observed in the presence of strychnine. Interestingly, glycine and taurine appeared to generate neuroprotective effects only at their optimum concentration range. By contrast, blockade of GlyT1 by *N*-[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl]sarcosine or sarcosine did not elicit significant neuroprotection. These results suggest that activation of strychnine-sensitive glycine receptors potentially produces neuroprotection against metabolic stress such as OGD. However, GlyT1 inhibition is unlikely to elicit a sufficient increase in the extracellular level of glycine to generate neuroprotection.

Keywords: oxygen/glucose deprivation, neuroprotection, glycine, strychnine-sensitive glycine receptor, glycine transporter

Introduction

The excitatory synaptic transmission or population spike recorded in brain slices in vitro has been widely employed in electrophysiological studies to assess the neuroprotective properties of drugs in simulated hypoxic/ischemic conditions. For instance, introduction of metabolic stress such as oxygen/glucose deprivation (OGD) in the CA1 pyramidal region of the hippocampus results in an initial decrease of the excitatory synaptic transmis-

sion and population spike due to an increase in the extracellular concentration of adenosine, which is considered to represent an endogenous neuroprotective mechanism (1 – 4). In a longer period of energy deprivation, however, the initial decrease of the excitatory synaptic transmission and population spike is followed by their irreversible loss with no apparent post-hypoxic/ischemic recovery. It appears that the endogenous neuroprotective mechanism mediated via adenosine is overcome by glutamate-induced neuronal hyperexcitability (5). Therefore, pharmacological manipulations that can potentiate inhibitory mechanisms may lead to reduced neuronal activity with lowered energy consumption and hamper the excitotoxic effects of glutamate.

Recent lines of evidence indicate that strychnine-sensitive glycine receptors are expressed functionally in the

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hippocampus (6–8), suggesting that an increase of glycine might have a neuroprotective effect against post-hypoxic/ischemic injury. However, glycine acts also as a co-agonist of glutamate to facilitate neuronal excitation mediated by NMDA receptors (9), and therefore the net influence of an increased extracellular glycine level on post-hypoxic/ischemic injury has yet to be elucidated. In the study presented here, excitatory synaptic transmission was recorded in the stratum radiatum of the CA1 area in mouse hippocampal slices *in vitro*, and the effect of exogenously applied glycine and taurine, which are both strychnine-sensitive glycine-receptor agonists, on its restoration after termination of OGD was evaluated.

The extracellular concentration of glycine is regulated by its reuptake via sodium/chloride-dependent glycine transporters (GlyTs) (10, 11). Although two GlyT subtypes, GlyT1 and GlyT2, have been identified, the presence of GlyT2 has been demonstrated in axons and pre-synaptic terminals of inhibitory glycinergic neurons in lower brain regions including the brainstem, cerebellum, and spinal cord (12, 13). By contrast, GlyT1, expressed widely in the central nervous system (14) and localized mostly in glial cells (13, 15), reduces the concentration of glycine near NMDA receptors where glycine acts as a co-agonist of glutamate to facilitate excitatory synaptic transmission mediated by NMDA receptors (16–18). In the hippocampus, an increase of endogenous glycine after blockade of GlyT1 has been shown to reduce neuronal firing via strychnine-sensitive glycine receptors (6), suggesting that the inhibitory effect could overcome the NMDA receptor-mediated excitation. In this study, therefore, the effect of an endogenous increase of glycine due to blockade of GlyT1 with sarcosine and *N*-[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl]sarcosine (NFPS), both of which are GlyT inhibitors, on the post-ischemic recovery of excitatory synaptic transmission was also evaluated.

Materials and Methods

All of the experimental protocols used in the present study were approved by the Animal Care and Use Committee of Nagoya City University and carried out in accordance with the guidelines of the National Institutes of Health and The Japanese Pharmacological Society.

Slice preparation and electrophysiological recordings

Five-week-old male ddY-strain mice (SLC, Shizuoka) were killed by cervical dislocation under anesthesia with ether. The brain was quickly removed and placed in ice-cold low-sodium artificial cerebrospinal fluid (low-sodium aCSF, pH 7.4 after bubbling with 95% O₂ and 5% CO₂) containing 212.5 mM sucrose, 3.5 mM KCl, 1.24

mM KH₂PO₄, 26 mM NaHCO₃, 10 mM glucose, 2.0 mM CaCl₂, and 1.0 mM MgSO₄. After the hippocampus had been isolated, transverse slices (400- μ m-thick) were prepared using a vibratome (DSK-1000; Dosaka, Kyoto) and then maintained for at least 60 min in standard aCSF (at 32°C, pH 7.4, after bubbling with 95% O₂ and 5% CO₂) containing 124 mM NaCl, 2 mM KCl, 1.24 mM KH₂PO₄, 26 mM NaHCO₃, 10 mM glucose, 2.0 mM CaCl₂, and 1.0 mM MgSO₄. The slices were then transferred to a recording chamber (chamber volume, approximately 0.5 ml) and superfused with standard aCSF kept at 32°C. Each slice was completely submerged in the medium and superfused continuously at a rate of 2.5 ml/min. The Schaffer collateral/commissural fibers were stimulated with square-wave pulses (0.1-ms duration at 10-s intervals) via a bipolar enamel-coated stainless steel electrode, and the field excitatory postsynaptic potentials (fEPSPs) were recorded with glass electrodes (1–5 M Ω) filled with 2 M NaCl, placed in the CA1 stratum radiatum. The stimulus intensity was adjusted so that 50% of the maximal response was elicited. fEPSPs were acquired and their initial slopes were measured using the Measurement and Analysis System for Extracellular Potentials (Furusawa Lab Appliance, Kawagoe).

OGD and drugs

OGD was induced by bath-applying aCSF containing no glucose and saturated with 95% N₂ + 5% CO₂ for 6 min. Each slice was exposed to only one period of OGD. Glycine and taurine were purchased from Nacalai Tesque (Kyoto) and Tokyo Chemical Industry (Tokyo), respectively. Strychnine hydrochloride and sarcosine were from Sigma Chemical Co. (St. Louis, MO, USA), and NFPS was from Tocris (Bristol, UK). NFPS was dissolved in dimethylsulfoxide (DMSO) to give a final solvent concentration of 0.1%. The other drugs were dissolved in distilled water. They were applied directly via the superfusion solution for 10 min (all drugs except for strychnine) or 20 min (strychnine) before, and then continuously throughout the subsequent OGD and reperfusion periods.

Statistics

The initial slopes of fEPSPs were normalized to the averaged slope of 30–60 consecutive fEPSPs obtained during 5–10 min before induction of OGD. All data are expressed as means $\pm$ S.E.M. Student's *t*-test (two-tailed) was used to compare the data for two groups. The two-tailed *t*-test with Bonferroni correction following one-way analysis of variance (ANOVA) was used for multiple comparisons between the control and the treated groups (19). Differences at *P* < 0.05 were considered significant.

Results

Time-course changes in the fEPSP slope in response to OGD

The pattern of changes in the fEPSP slope in response to OGD was consistent with previous studies reported by others (20, 21). As shown in Fig. 1, after introduction of OGD, fEPSPs were initially inhibited and the presynaptic volley (PV) was unchanged (Fig. 1: b – c), followed by transient recovery of fEPSPs for a few minutes (Fig. 1d). The transient recovery was observed in each slice, although its peak amplitude varied with the slice. Then, fEPSPs and therefore their slope were completely depressed with disappearance of the PV around 6 min after initiation of OGD (Fig. 1e: 12 min after initiation of OGD). Under our experimental conditions, fEPSPs were potentially restored when the slice was reperfused with normal aCSF saturated with 95% O₂ and 5% CO₂ before disappearance of the PV. However, when the slice was exposed to OGD conditions even after disappearance of the PV, fEPSPs remained irreversibly lost even 60 min after reperfusion (data not shown). Therefore in the present study, each slice was always exposed to OGD conditions for 6 min, which allowed the fEPSP slope to be restored to about 10% of that obtained before initiation of OGD 40 min after reperfusion (Fig. 1f).

Fig. 1. A representative time course of changes in the fEPSP slope during and after oxygen/glucose deprivation (OGD) in the CA1 stratum radiatum of a mouse hippocampal slice. OGD was introduced by bath-applying aCSF containing no glucose and saturated with 95% N₂ + 5% CO₂ for 6 min. The changes in the fEPSP slope were expressed as percentages relative to the averaged slope of 30 consecutive fEPSPs obtained during 5 min before induction of OGD. Inset: Example traces of the fEPSP recorded at the times indicated on the graph.

Neuroprotective effect of bath-applied glycine assessed during post-ischemic recovery of excitatory synaptic transmission

Bath-applied glycine (0.1 and 0.3 mM) alone did not elicit apparent reduction of the fEPSP slope. However, 1 mM glycine reduced the fEPSP slope by $18.2 \pm 4.8\%$ ($n = 5$, $P < 0.05$) during 10 min before initiation of OGD (Fig. 2A). In the presence of strychnine, 1 mM glycine never decreased the fEPSP slope, indicating that this reduction was mediated by strychnine-sensitive glycine receptors (data not shown). The restoration of the fEPSP slope after reperfusion was facilitated only when glycine was perfused at 0.3 mM (Fig. 2: A, C, and D). By contrast, at lower (0.1 mM) or higher (1 mM) concentrations, the restoration of the fEPSP slope was rather inhibited, and fEPSPs remained almost lost. The fEPSP slopes 40 min after reperfusion were $12.2 \pm 7.5\%$ ($n = 15$), $1.6 \pm 1.0\%$ ($n = 6$), $53.7 \pm 13.1\%$ ($n = 12$, $P < 0.01$ vs. control), and 0% ($n = 5$) for the control and glycine concentrations of 0.1, 0.3 and 1 mM, respectively (Fig. 2D). Since the fEPSP slope indicated no promotion of post-ischemic recovery when 0.3 mM glycine was applied in the presence of 1 μ M strychnine (Fig. 2: B – D, $4.3 \pm 2.5\%$, $n = 5$, $P < 0.05$ vs. 0.3 mM glycine alone), it was suggested that glycine produces neuroprotective effects via strychnine-sensitive glycine receptors.

Neuroprotective effect of bath-applied taurine assessed during post-ischemic recovery of excitatory synaptic transmission

In slices perfused with taurine (0.3, 1, and 3 mM), we obtained results similar to those with glycine. Again, bath-applied taurine (0.3 and 1 mM) alone did not elicit any apparent reduction of the fEPSP slope. However, 3 mM taurine reduced the fEPSP slope by $21.1 \pm 7.2\%$ ($n = 6$, $P < 0.05$) during 10 min before initiation of OGD (Fig. 3A). The restoration of the fEPSP slope after reperfusion was facilitated only when taurine was perfused at 1 mM (Fig. 3: A, C, and D). By contrast, at lower (0.3 mM) or higher (3 mM) concentrations, the restoration of the fEPSP slope was not different from the control group (0.3 mM) or rather inhibited (3 mM). The fEPSP slopes 40 min after reperfusion were $13.4 \pm 7.4\%$ ($n = 16$), $20.1 \pm 18.3\%$ ($n = 5$), $54.0 \pm 14.5\%$ ($n = 11$, $P < 0.05$ vs. control), and $5.0 \pm 3.7\%$ ($n = 6$) for the control and taurine concentrations of 0.3, 1, and 3 mM, respectively (Fig. 3D). Since the fEPSP slope did not show any promotion of post-ischemic recovery when 1 mM taurine was applied in the presence of 1 μ M strychnine (Fig. 3: B – D, $8.3 \pm 6.5\%$, $n = 6$, $P < 0.05$ vs. 1 mM taurine alone), it was suggested that taurine produces neuroprotective effects via strychnine-sensitive glycine receptors.

Fig. 2. Effects of glycine on the post-ischemic recovery of excitatory synaptic transmission. A and B: Time-course graphs depicting changes in the fEPSP slope during and after OGD in the presence or absence of glycine (A, gly; 0.1, 0.3, and 1 mM), and the effect of glycine (0.3 mM) in the presence of strychnine (B, 1 μ M). Glycine and strychnine were superfused for 10 and 20 min beforehand and then continuously throughout the subsequent OGD and reperfusion periods, respectively. The changes in the fEPSP slope were expressed as percentages relative to the averaged slope of 30–60 consecutive fEPSPs obtained during 5–10 min before induction of OGD. All the data are expressed as means $\pm$ S.E.M. ($n = 5–15$). C: Example traces of the fEPSP recorded before OGD and 40 min after the start of reperfusion (broken line). D: Summary of the neuroprotective effect of glycine and its blockade by strychnine. Ordinates: the fEPSP slope 40 min after the start of reperfusion, expressed as means $\pm$ S.E.M. ($n = 5–15$). Two-tailed t -test with Bonferroni correction following one-way analysis of variance (ANOVA) was used for multiple comparisons between the control and glycine-treated groups and between the glycine (0.3 mM)-treated and strychnine-pretreated groups (four comparisons in five groups, $*P < 0.05$ and $**P < 0.01$).

No neuroprotective effect of endogenous glycine under blockade of GlyT1

We first employed the non-competitive GlyT1 inhibitor NFPS (22, 23) to assess whether endogenously increased glycine promotes post-ischemic recovery of excitatory synaptic transmission. NFPS was used at concentrations up to 100 nM, which has been demonstrated to produce effective blockade of GlyT1, as evidenced by enhanced NMDA receptor-mediated excitatory synaptic currents (16, 17). As demonstrated in Fig. 4, however, post-ischemic recovery of excitatory synaptic transmission was never facilitated and in fact, was somewhat hampered at higher concentrations (30 and

100 nM). The fEPSP slopes 40 min after reperfusion were $16.1 \pm 9.3\%$ ($n = 15$), $18.8 \pm 15.8\%$ ($n = 6$), $0.7 \pm 0.7\%$ ($n = 5$), and $8.2 \pm 3.1\%$ ($n = 5$) for the control and NFPS concentrations of 10, 30, and 100 nM, respectively (Fig. 4C).

We then evaluated the neuroprotective effect of the competitive GlyT1 inhibitor sarcosine, which acts as a substrate (11, 22). Although sarcosine (0.3 and 1 mM) alone did not elicit any apparent reduction of the fEPSP slope, 3 mM sarcosine reduced the fEPSP slope by $23.9 \pm 10.0\%$ ($n = 5$, $P > 0.05$) during 10 min before initiation of OGD (Fig. 5A). Sarcosine at 1 mM tended to restore the fEPSP slope after reperfusion (not signifi-

Fig. 3. Effects of taurine on the post-ischemic recovery of excitatory synaptic transmission. **A** and **B**: Time-course graphs depicting changes in the fEPSP slope during and after OGD in the presence or absence of taurine (**A**, tau; 0.3, 1, and 3 mM), and the effect of taurine (1 mM) in the presence of strychnine (**B**, 1 μ M). Taurine and strychnine were superfused for 10 and 20 min beforehand and then continuously throughout the subsequent OGD and reperfusion periods, respectively. The changes in the fEPSP slope were expressed as percentages relative to the averaged slope of 30–60 consecutive fEPSPs obtained during 5–10 min before induction of OGD. All the data are expressed as means $\pm$ S.E.M. ($n = 5–16$). **C**: Example traces of the fEPSP recorded before OGD and 40 min after the start of reperfusion (broken line). **D**: Summary of the neuroprotective effect of taurine and its blockade by strychnine. Ordinates: the fEPSP slope 40 min after the start of reperfusion, expressed as means $\pm$ S.E.M. ($n = 5–16$). Two-tailed *t*-test with Bonferroni correction following one-way analysis of variance (ANOVA) was used for multiple comparisons between the control and taurine-treated groups and between the taurine (1 mM)-treated and strychnine-pretreated groups (four comparisons in five groups, $*P < 0.05$).

cant, see below), whereas at lower (0.3 mM) or higher (3 mM) concentrations, the restoration of the fEPSP slope was somewhat inhibited. The fEPSP slopes 40 min after reperfusion were $5.8 \pm 4.7\%$ ($n = 17$), $1.1 \pm 1.1\%$ ($n = 6$), $31.8 \pm 20.3\%$ ($n = 5$, $P > 0.05$ vs. control), and $2.3 \pm 1.7\%$ ($n = 5$) for the control and sarcosine concentrations of 0.3, 1, and 3 mM, respectively (Fig. 5C).

Discussion

A number of attempts have been made to counteract post-hypoxic/ischemic injury by increasing inhibitory transmission. However, such studies have usually fo-

cused on the GABAergic inhibition, and pharmacological manipulations leading to activation of GABA receptors, including GABA_A and GABA_B receptors, indeed generate neuroprotective effects against energy deprivation (24–27). By contrast, despite functional expression of strychnine-sensitive glycine receptors in the frontal cortical/hippocampal brain areas, few studies have demonstrated neuroprotective effects of glycine, another major inhibitory transmitter, under hypoxic/ischemic conditions (28). Rather, most studies have described its neurotoxic effects in relation to glutamate, excessive accumulation of which caused by hypoxia/ischemia triggers cellular processes leading to neuronal injury. In the pres-

Fig. 4. Effects of NFPS on the post-ischemic recovery of excitatory synaptic transmission. A: Time-course graph depicting changes in the fEPSP slope during and after OGD in the presence or absence of NFPS (10, 30, and 100 nM). NFPS was superfused for 10 min beforehand and then continuously throughout the subsequent OGD and reperfusion periods. The changes in the fEPSP slope were expressed as percentages relative to the averaged slope of 30 consecutive fEPSPs obtained during 5 min before induction of OGD. All the data are expressed as means $\pm$ S.E.M. ($n = 5 - 15$). B: Example traces of the fEPSP recorded before OGD and 40 min after the start of reperfusion (broken line). C: Summary of the effect of NFPS. Ordinates: the fEPSP slope 40 min after the start of reperfusion, expressed as means $\pm$ S.E.M. ($n = 5 - 15$).

ent study, we recorded fEPSPs in the stratum radiatum of the CA1 area in mouse hippocampal slices and evaluated their post-ischemic recovery, which has been demonstrated to correlate well with morphologically assessed neuronal survival (29). Our study clearly demonstrated that glycine (and taurine) applied exogenously, but not an endogenous increase of glycine induced by blockade of GlyT1, exerted neuroprotective effects against OGD by activating strychnine-sensitive glycine receptors. However, the neuroprotection mediated via strychnine-sensitive glycine receptors was biphasic; concentrations of glycine and taurine lower and higher than their optimum concentration elicited no significant protection, and the post-ischemic recovery of the fEPSPs was sometimes rather hampered at their higher concentrations.

Glycine may hyperpolarize hippocampal pyramidal neurons by gating chloride channels coupled with strychnine-sensitive glycine receptors (6 - 8). Moreover, an increase in chloride conductance in CA1 pyramidal neurons may shunt and therefore reduce glutamatergic excitatory synaptic potentials, thus preventing the generation of action potentials (8). These effects mediated by

strychnine-sensitive glycine receptors should inhibit hippocampal neuronal activity, which appears to have made a considerable contribution to the neuroprotection observed in the present study. Our study may partly support that of Wang et al. (30) demonstrating the dose-dependent neuroprotective effect of systemically administered taurine against post-ischemic injury through activation of GABA_A and strychnine-sensitive glycine receptors. However, as the present results showed, glycine and taurine appeared to have their optimum concentration range to generate neuroprotective effects, and glycine and taurine of higher concentrations (1 and 3 mM, respectively), which alone reduced the initial slope of fEPSPs presumably through a decrease of input resistance due to an increase of chloride conductance, did not elicit any promotion of post-ischemic fEPSPs recovery. It has been demonstrated that an excessive influx of chloride ions via strychnine-sensitive glycine receptors potentiates NMDA-induced neurotoxicity in rat cortical cultures (31). It appears that activation of strychnine-sensitive glycine receptors under the excessive rise in intracellular chloride ions elicits depolarization, leading

Fig. 5. Effects of sarcosine on the post-ischemic recovery of excitatory synaptic transmission. A: Time-course graph depicting changes in the fEPSP slope during and after OGD in the presence or absence of sarcosine (0.3, 1, and 3 mM). Sarcosine was superfused for 10 min beforehand and then continuously throughout the subsequent OGD and reperfusion periods. The changes in the fEPSP slope were expressed as percentages relative to the averaged slope of 30–60 consecutive fEPSPs obtained during 5–10 min before induction of OGD. All the data are expressed as means $\pm$ S.E.M. ($n = 5–17$). B: Example traces of the fEPSP recorded before OGD and 40 min after the start of reperfusion (broken line). C: Summary of the effect of sarcosine. Ordinates: the fEPSP slope 40 min after the start of reperfusion, expressed as means $\pm$ S.E.M. ($n = 5–17$).

to facilitation of NMDA-induced neurotoxicity. Neurotoxicity induced by excessive loading of chloride ions via the GABA_A–chloride channel complex has also been suggested by a similar bell-shaped dose–response relationship of GABA during the post-ischemic recovery of field potential amplitude in striatal neurons (25). Therefore, it is most likely that activation of strychnine-sensitive glycine receptors elicits neuroprotection in the hippocampal pyramidal neurons as long as the inhibitory effect on neuronal activity overcomes the neurodegenerative effect triggered by an overload of chloride ion into the neurons.

In addition to the effects exerted via strychnine-sensitive glycine receptors, glycine, but not taurine, exerts excitatory influences on neuronal activity as a co-agonist of glutamate at NMDA receptors (9), thus counteracting the neuroprotective effect of glycine, as supported by a number of studies that have demonstrated the neurotoxic effect of glycine mediated by NMDA receptors (32–35). Moreover, ischemic conditions elicit an increase in the extracellular concentration of glycine (36, 37), which has been shown to contribute to the ischemic injury involv-

ing NMDA receptors (37). Therefore, under the present experimental conditions whereby the slice was exposed to OGD in the continuous presence of exogenously applied glycine, the post-ischemic injury may possibly have been exaggerated.

In the hippocampus, extracellular glycine is regulated primarily by its reuptake via GlyT1 expressed close to NMDA receptors (13). It has recently been demonstrated that endogenously increased glycine after blockade of GlyT1 can reach strychnine-sensitive glycine receptors to exert inhibitory influences on neuronal activity in the hippocampal CA1 region (6, 38). However, we were unable to elicit significant neuroprotective effects in slices exposed to OGD under blockade of GlyT1. It is likely that such blockade would not increase the extracellular level of glycine near strychnine-sensitive glycine receptors sufficiently to overcome the excitation elicited by activation of NMDA receptors. Sarcosine exhibited a weak neuroprotective effect characterized by a bell-shaped-like concentration–response relationship, similar to that of glycine and taurine. However, NFPS did not exert any neuroprotective effects even at a concentration

of 100 nM, which has been demonstrated to increase NMDA receptor-mediated synaptic transmission by blocking the uptake of transportable endogenous glycine (17). Sarcosine and NFPS are transportable and non-transportable inhibitors of GlyT1, respectively (22), and therefore sarcosine may increase the extracellular level of glycine not only by blockade of glycine uptake via GlyT1 but also heteroexchange of glycine via GlyT1. Moreover, a recent study by Zhang et al. (39) has revealed that sarcosine is capable of acting as an agonist of strychnine-sensitive glycine receptors. Therefore, it appears that recruitment of extracellularly available glycine alone by blockade of its uptake via GlyT1 is not enough to generate neuroprotective effects against post-ischemic injury. Since the extracellular concentration of taurine is also regulated by its specific transporter (40), it would be of interest to assess whether pharmacological blockade of its transport system can promote post-ischemic injury without activating NMDA receptors as a co-agonist of glutamate. This should be assessed in a future study.

In conclusion, we have demonstrated that stimulation of strychnine-sensitive glycine receptors is able to generate neuroprotective effects by assessing its effect on the post-ischemic functional recovery of excitatory synaptic transmission in the hippocampus. Although excessive activation of these receptors appears to be neurotoxic, we propose that a combination of lower doses of glycine or taurine and other neuroprotective drugs with different mechanisms might elicit better neuroprotection with fewer adverse effects, thus providing a novel and clinically relevant combination therapy.

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