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The Glycine Transport Inhibitor Sarcosine Is an Inhibitory Glycine Receptor Agonist

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Summary

Sarcosine is an endogenous amino acid that is a competitive inhibitor of the type I glycine transporter (GlyT1), an N-methyl-D-aspartate receptor (NMDAR) co-agonist, and an important intermediate in one-carbon metabolism. Its therapeutic potential for schizophrenia further underscores its clinical importance. The structural similarity between sarcosine and glycine and sarcosine's ability to serve as an NMDAR co-agonist led us to examine whether sarcosine is also an agonist at the inhibitory glycine receptor (GlyR). We examined this possibility using whole-cell recordings from cultured embryonic mouse hippocampal neurons and found that sarcosine evoked a dose-dependent, strychnine sensitive, Cl^- current that cross-inhibited glycine currents. Sarcosine evoked this current with Li^+ in the extracellular solution to block GlyT1, in neurons treated with the essentially irreversible GlyT1 inhibitor *N*[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl]sarcosine (NFPS), and in neurons plated in the absence of glia. These results indicate that the sarcosine currents did not result from GlyT1 inhibition or heteroexchange. We conclude that sarcosine is a GlyR agonist.

Six Keywords

culture; schizophrenia; hippocampus; N-methyl-D-aspartate; *N*[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl]sarcosine; patch clamp

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Introduction

Sarcosine (N-methylglycine, Fig. 1A) is an important intermediate in one-carbon metabolism (Ueland et al., 2007), a competitive inhibitor of the type I glycine transporter (GlyT1) (Smith et al., 1992; Lopez-Corcuera et al., 1998; Herdon et al., 2001; Mallorga et al., 2003), and an N-methyl-D-aspartate receptor (NMDAR) co-agonist (Zhang et al., 2009). One-carbon metabolism refers to the folate dependent pathways involved in activating single carbons for protein synthesis, nucleotide synthesis, and DNA methylation. GlyT1 is located primarily on glia and helps determine the glycine concentration available to activate NMDARs (Eulenburg et al., 2005). As a GlyT1 inhibitor and an NMDAR co-agonist, sarcosine can enhance NMDAR function, which may be low in schizophrenia. Accordingly, sarcosine and other potentiators of NMDAR function appear effective in treating schizophrenia (Shim et al., 2008; Javitt, 2009).

Sarcosine may improve the symptoms of schizophrenia because it is a GlyT1 inhibitor and NMDAR co-agonist, but it may have other effects that are important to consider clinically. Specifically, the endogenous amino acids sarcosine and glycine differ by a methyl group giving sarcosine the potential to be an inhibitory glycine receptor (GlyR) agonist. We have shown that sarcosine is a NMDAR co-agonist at slightly lower concentrations than it is a GlyT1 antagonist (Zhang et al., 2009) (Table 1). Here we show that it is a GlyR agonist at higher concentrations using whole-cell voltage clamp recordings from cultured embryonic mouse hippocampal neurons. We have published a preliminary version of this work (Zhang and Thio, 2008).

Methods

Embryonic mouse hippocampal cultures

The Washington University Animal Studies Committee approved all experimental protocols, which were performed in accordance with guidelines published by the NIH and in the *Guide for the Care and Use of Laboratory Animals*. Every effort was made to minimize animal suffering and to reduce the number of animals used. Cultured hippocampal neurons were obtained from Swiss Webster mouse embryos at day 16 of gestation as described previously (Thio et al., 2003; Zhang and Thio, 2007). Timed pregnant mice were sacrificed by deep anesthesia with isoflurane followed by cervical dislocation. Hippocampal slices were enzymatically digested with papain to generate a single cell suspension, which then was plated on a monolayer of cortical astrocytes. Glial free neuronal cultures were obtained by plating neurons directly on poly-L-lysine coated coverslips lacking an astrocytic monolayer. Most experiments were performed using neurons cultured for 7-9 days, though cultures ranging from 5-16 days were used.

Electrophysiology

Whole-cell patch clamp electrophysiology—Whole-cell GlyR mediated currents were recorded at room temperature using an Axopatch 200A amplifier (Molecular Devices, Sunnyvale, CA) as described previously (Thio et al., 2003; Zhang and Thio, 2007). Voltage-clamp recordings were obtained at a holding potential of -65 mV unless otherwise indicated. Current-voltage plots were obtained by subjecting neurons to voltage ramps of 0.1 V/s. All holding potentials were corrected for empirically measured junction potentials. Series resistance compensation was set at 60-90%. Currents were low pass filtered at 2 kHz using the 4-pole low pass filter on the amplifier and digitized at 10 kHz using pCLAMP 9 (Molecular Devices).

Solutions—The extracellular solution contained (in mM): 140 NaCl, 5 KCl, 1.5 CaCl₂, 1 MgCl₂, 10 D-glucose, 2.5×10^{-4} tetrodotoxin (TTX), and 10 N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES) (pH 7.35 - 7.39). The patch pipettes had resistances of 2-6 MΩ and were filled with a solution containing (in mM): 140 CsCl, 4 NaCl, 0.5 CaCl₂, 5 ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 0.5 Na₃GTP, 2 MgATP, and 10 HEPES (pH 7.20 - 7.30). Equimolar Cs methanesulfonate (CsCH₃SO₃) replaced the CsCl for some voltage ramp experiments.

Drug application—A multibarrel, gravity-driven, flow tube system was used to apply agonists and antagonists at 2 ml/min as described previously (Thio et al., 2003; Zhang and Thio, 2007). Antagonists were pre-applied for at least 60 s. The neuron being studied was continuously perfused with extracellular solution alone between drug applications. At all times, the recording chamber was perfused with extracellular solution at 0.5 ml/min. This system floods the cell with the agonists and antagonists of interest without allowing them to accumulate over time. This design also does not allow uptake systems to decrease their concentrations during an application.

Data analysis—Current traces were analyzed using pCLAMP 9. Control and experimental applications generally were interleaved, and the data were not used if the bracketing control peak currents were not within 10-15% of each other. Using this criterion, typically two to three trials from each neuron were analyzed.

Sarcosine dose-response curves were fit to the logistic equation

$$R(\text{Sarcosine}) = \frac{R_{\max} \times [\text{Sarcosine}]^N}{EC_{50}^N + [\text{Sarcosine}]^N} \quad (1)$$

where $R(\text{Sarcosine})$ is the response to a given sarcosine concentration $[\text{Sarcosine}]$, $R_{\max}$ is the response to a saturating sarcosine concentration, EC_{50} is the sarcosine concentration producing a half-maximal response, and N is the Hill coefficient.

Strychnine dose-response curves were fit to the logistic equation

$$R(\text{Strychnine}) = \frac{R_{\text{Sarcosine}} \times IC_{50}^N}{IC_{50}^N + [\text{Strychnine}]^N} \quad (2)$$

where $R(\text{Sarcosine})$ is the response to sarcosine in the presence of a given strychnine concentration $[\text{Strychnine}]$, $R_{\text{Sarcosine}}$ is the response to sarcosine in the absence of strychnine, IC_{50} is the strychnine concentration producing half-maximal inhibition, and N is the Hill coefficient. Fits were obtained using the Levenberg-Marquardt algorithm.

Statistics

Statistical analysis was performed using Origin 7 (OriginLab, Northampton, MA), and Microsoft Excel 2000 (Microsoft, Redmond, WA). Data are presented as the mean $\pm$ standard error with n being the number of neurons studied. Error bars smaller than symbols are not shown. Means were compared using a two-tailed paired t-test with significance set at $p < 0.05$.

Materials

All chemicals were obtained from Sigma (St. Louis, MO) except for *N*[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl]sarcosine (NFPS), which was obtained from Tocris Bioscience (Ellisville, MO).

Results

As expected of a GlyR mediated current, sarcosine alone evoked a dose-dependent, Cl^- current with an EC_{50} of 3.2 ± 0.7 mM ($n = 11$) and an N of 1.5 ± 0.2 ($n = 11$) (Fig. 1B and C). Sarcosine was less potent than glycine, which has an EC_{50} of 60 μM in this preparation (Thio et al., 2003). Three mM sarcosine activated a Cl^- conductance because the reversal potential was -65 ± 3 mV ($n = 6$) using the CsMeSO_3 pipette solution and -7 ± 1 mV ($n = 5$) using the CsCl pipette solution (Fig. 1D). We determined the reversal potential by applying voltage ramps during 3 mM sarcosine currents showing no decline during a two second application (Fig. 1B and D). In the neurons selected, the sarcosine current amplitude after the ramp was $100 \pm 4\%$ ($n = 11$) of the amplitude before the ramp (Fig. 1D inset).

We performed two types of experiments to demonstrate that sarcosine activates GlyRs. First, the GlyR inhibitor strychnine inhibited 3 mM sarcosine currents with an IC_{50} of 17 ± 3 nM ($n = 12$) and an N of 1.1 ± 0.1 ($n = 12$) (Fig. 2A and B), which is similar to its potency against glycine (Thio et al., 2003). To provide further evidence that sarcosine activates GlyRs, we examined the interaction between currents evoked by saturating concentrations of sarcosine (10 mM) and glycine (300 μM) (Thio et al., 2003). We noted that 10 mM sarcosine peak currents were only $75 \pm 5\%$ ($n = 8$, $p = 0.003$ by two-tailed paired t-test) of 300 μM glycine peak currents suggesting that sarcosine is not a full agonist. Co-applying 10 mM sarcosine and 300 μM glycine produced a peak current that was $55 \pm 2\%$ ($n = 8$, $p = 0.004$ by two-tailed paired t-test) of the arithmetic sum of the individual responses (Fig. 2C). In addition, 10 mM sarcosine and 300 μM glycine peak currents showed cross-inhibition. Applying 300 μM glycine during a 10 mM sarcosine steady-state current produced a peak glycine current that was $27 \pm 10\%$ ($n = 7$, $p = 0.009$ by two-tailed paired t-test) of control (Fig. 2D). Conversely, applying 10 mM sarcosine during a 300 μM glycine steady-state current did not produce a detectable sarcosine current ($1 \pm 1\%$ control, $n = 7$, $p = 0.009$ by two-tailed paired t-test) (Fig. 2E). Together, these findings indicate that sarcosine activates GlyRs.

Sarcosine may evoke strychnine sensitive glycine currents by directly binding and gating GlyRs or indirectly by causing glycine to accumulate in the extracellular solution. This accumulation may result from the block of glycine uptake via GlyT1 or by heteroexchange of sarcosine for glycine via GlyT1 (Herdon et al., 2001). Our difficulty recording GlyT1 currents from astrocytes suggests that GlyT1 activity is low under our experimental conditions. However, we elected to exclude this possibility formally by using the GlyT1 inhibitors NFPS and Li^+ . In five neurons exhibiting a sarcosine current, 1 μM NFPS elicited no current (Fig. 3A). In these neurons, the 3 mM sarcosine peak current after applying 1 μM NFPS for 10 min was $98 \pm 5\%$ ($n = 5$, $p = 0.3$ by two-tailed paired t-test) of the control peak current obtained before applying NFPS. Furthermore, sarcosine evoked currents in 8 of 9 neurons from cultures treated with 1 μM NFPS for 60 min prior to obtaining whole-cell recordings. The peak currents evoked by 3 mM sarcosine in these neurons were 1600 ± 360 pA (range 500 – 3300 pA, $n = 8$). The 3 mM sarcosine peak current was $93 \pm 7\%$ ($n = 5$, $p = 0.3$ by two-tailed paired t-test) of control after replacing all the extracellular sodium with Li^+ . Next, we opted to determine whether sarcosine elicited a current in the absence of GlyT1, which we achieved by using glial free cultures. In all such neurons tested, 3 mM sarcosine evoked a current having an amplitude of 1400 ± 220 pA ($n = 5$) (Fig. 3B). These results suggest that sarcosine activates GlyRs via a GlyT1 independent mechanism.

Other potential sources of glycine include sarcosine itself, background levels in the cultures, or synaptic release. Our sarcosine stock contains 0.001% glycine (Zhang et al., 2009), but the contaminating glycine concentrations in our sarcosine solutions are insufficient to activate GlyRs in this preparation (Thio et al., 2003). As we reported previously, the background concentration of glycine in the cultures under our experimental conditions is 20 nM (Zhang et al., 2009). This concentration is insufficient to activate GlyRs. In principle, sarcosine could cause synaptic release of glycine by depolarizing glycinergic neurons in the culture. Assuming a sufficient background glutamate concentration, the depolarization could result from sarcosine acting as an NMDAR co-agonist as we recently reported (Zhang et al., 2009). This mechanism probably does not occur because 100 μ M sarcosine, the lowest sarcosine concentration examined here, elicited no current. This sarcosine concentration saturates the NMDAR mediated response (Zhang et al., 2009) and should have elicited a GlyR mediated current according to this hypothesis. In addition, we have not observed GlyR mediated synaptic responses in our cultures (Thio and Yamada, 2004). We conclude that sarcosine itself activates GlyRs.

Discussion

The principal finding of this study is that sarcosine is a GlyR agonist. We conclude that sarcosine activates GlyRs because it evoked a strychnine sensitive, dose-dependent, Cl^- current. The lack of additivity with glycine currents and the reciprocal cross-inhibition with glycine currents supports this conclusion. Importantly, we obtained the current with Li^+ in the extracellular solution, in neurons treated with the essentially irreversible GlyT1 inhibitor NFPS, and in neurons grown in the absence of glia to eliminate GlyT1. These results exclude an increase in extracellular glycine via GlyT1 inhibition or GlyT1 mediated heteroexchange from being the mechanism by which sarcosine activates GlyR mediated currents. Thus, sarcosine is a GlyR agonist, an NMDAR co-agonist, and a GlyT1 inhibitor, though it is least potent as a GlyR agonist (Table 1).

The finding that sarcosine is a GlyR agonist in addition to being a NMDAR co-agonist is not surprising given its structural similarity to glycine. Other transport inhibitors such as guanidinoethyl sulphonate (Mellor et al., 2000; Sergeeva et al., 2002) and dihydrokainate (Thio et al., 1991; Arriza et al., 1994) interact with their respective receptors. However, sarcosine is less potent than glycine as a GlyR agonist and is not a full agonist. These differences may result from the additional methyl group on sarcosine creating steric hindrance with the glycine binding site on the GlyR. The structural similarity raises the possibility that glycine actually evokes our sarcosine currents because sarcosine is demethylated to glycine in our cultures. This possibility seems unlikely because we used a flow tube system to perfuse the neuron studied continuously in a constantly perfused bath. In addition, sarcosine dehydrogenase, which demethylates sarcosine to form glycine, is difficult to detect in brain tissue (Bergeron et al., 1998) and therefore is unlikely to be present in our cultures.

Our findings indicate that identifying the mechanism underlying an experimental or clinical sarcosine effect may be difficult. Our findings are relevant to studies using sarcosine to inhibit GlyT1, which often use 500-750 μ M (Martina et al., 2004; Huang et al., 2004). Another study used sarcosine to determine whether endogenous glycine tonically activates hippocampal GlyRs (Zhang et al., 2008). The study found that 0.5 – 2 mM sarcosine induced strychnine sensitive currents in CA1 hippocampal pyramidal neurons. It also found that sarcosine inhibited pentylenetetrazole induced seizures. These findings might reflect GlyT1 inhibition, the direct activation of GlyRs by sarcosine, or both. Despite these studies using sarcosine concentrations below the EC_{50} for GlyR activation, small decreases in input resistance resulting from small amounts of GlyR activation are capable of altering hippocampal excitability (Zhang and Thio, 2007).

We speculate that the agonist effects of sarcosine at GlyRs have clinical relevance in some neuropsychiatric conditions. The beneficial effect of sarcosine in schizophrenia is thought to derive from enhanced NMDAR function whether by inhibiting GlyT1 or by acting as an NMDAR co-agonist (Tsai et al., 2004; Lane et al., 2005; Lane et al., 2008; Zhang et al., 2009). However, enhanced inhibition via GlyR activation may contribute to the clinical effect despite the need for higher sarcosine concentrations (Table 1). Presently, the sarcosine levels achieved in plasma and the brain using the doses prescribed in the published clinical studies are unknown. Enhanced inhibition may be helpful because γ -aminobutyric acid_A receptor (GABA_AR) function may be reduced in schizophrenia (Coyle, 2006). Clinically, GlyR activation, within and without the central nervous system (CNS), also could contribute to any adverse effects sarcosine may have when given to patients with schizophrenia. Although a rare and controversial condition, our results may have implications for sarcosinemia, which shows variable symptoms including cognitive impairment, growth retardation, hypertonia, and vomiting (Scott, 2001). These symptoms may result from excessive NMDAR activation as previously hypothesized (Deutsch et al., 2006), but excessive GlyR activation may also contribute as in nonketotic hyperglycinemia (Matalon et al., 1983).

In conclusion, sarcosine is a GlyR agonist in addition to being a GlyT1 inhibitor and NMDAR co-agonist. We have emphasized the CNS effects, but these actions may be relevant for other tissues such as the retina, which also use these neurotransmitter systems (Javitt, 2009). All three actions are important to consider whether using sarcosine as an experimental tool or a clinical therapy.

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References

- Arriza JL, Fairman WA, Wadiche JI, Murdoch GH, Kavanaugh MP, Amara SG. Functional comparisons of three glutamate transporter subtypes cloned from human motor cortex. *J Neurosci* 1994;14:5559–5569. [PubMed: 7521911]
- Bergeron F, Otto A, Blache P, Day R, Denoroy L, Brandsch R, Bataille D. Molecular cloning and tissue distribution of rat sarcosine dehydrogenase. *Eur J Biochem* 1998;257:556–561. [PubMed: 9839943]
- Coyle JT. Glutamate and schizophrenia: beyond the dopamine hypothesis. *Cell Mol Neurobiol* 2006;26:365–384. [PubMed: 16773445]
- Deutsch SI, Rosse RB, Long KD, Gaskins B, Mastropaolo J. Rare neurodevelopmental abnormalities of sarcosinemia may involve glycinergic stimulation of a primed N-methyl-d-aspartate receptor. *Clin Neuropharmacol* 2006;29:361–363. [PubMed: 17095900]
- Eulenburg V, Armsen W, Betz H, Gomeza J. Glycine transporters: essential regulators of neurotransmission. *Trends Biochem Sci* 2005;30:325–333. [PubMed: 15950877]
- Herdon HJ, Godfrey FM, Brown AM, Coulton S, Evans JR, Cairns WJ. Pharmacological assessment of the role of the glycine transporter GlyT-1 in mediating high-affinity glycine uptake by rat cerebral cortex and cerebellum synaptosomes. *Neuropharmacology* 2001;41:88–96. [PubMed: 11445189]
- Huang H, Barakat L, Wang D, Bordey A. Bergmann glial GlyT1 mediates glycine uptake and release in mouse cerebellar slices. *J Physiol* 2004;560:721–736. [PubMed: 15331688]
- Javitt DC. Glycine transport inhibitors for the treatment of schizophrenia: Symptom and disease modification. *Curr Opin Drug Discov Devel* 2009;12:468–478.
- Lane HY, Chang YC, Liu YC, Chiu CC, Tsai GE. Sarcosine or D-serine add-on treatment for acute exacerbation of schizophrenia: a randomized, double-blind, placebo-controlled study. *Arch Gen Psychiatry* 2005;62:1196–1204. [PubMed: 16275807]

- Lane HY, Liu YC, Huang CL, Chang YC, Liao CH, Perng CH, Tsai GE. Sarcosine (N-methylglycine) treatment for acute schizophrenia: a randomized, double-blind study. *Biol Psychiatry* 2008;63:9–12. [PubMed: 17659263]
- Lopez-Corcuera B, Martinez-Maza R, Nunez E, Roux M, Supplisson S, Aragon C. Differential properties of two stably expressed brain-specific glycine transporters. *J Neurochem* 1998;71:2211–2219. [PubMed: 9798949]
- Mallorga PJ, Williams JB, Jacobson M, Marques R, Chaudhary A, Conn PJ, Pettibone DJ, Sur C. Pharmacology and expression analysis of glycine transporter GlyT1 with [3H]-(N-[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl])sarcosine. *Neuropharmacology* 2003;45:585–593. [PubMed: 12941372]
- Martina M, Gorfinkel Y, Halman S, Lowe JA, Periyalwar P, Schmidt CJ, Bergeron R. Glycine transporter type 1 blockade changes NMDA receptor-mediated responses and LTP in hippocampal CA1 pyramidal cells by altering extracellular glycine levels. *J Physiol* 2004;557:489–500. [PubMed: 15064326]
- Matalon R, Naidu S, Hughes JR, Michals K. Nonketotic hyperglycinemia: treatment with diazepam--a competitor for glycine receptors. *Pediatrics* 1983;71:581–584. [PubMed: 6300746]
- Mellor JR, Gunthorpe MJ, Randall AD. The taurine uptake inhibitor guanidinoethyl sulphonate is an agonist at γ -aminobutyric acid_A receptors in cultured murine cerebellar granule cells. *Neurosci Lett* 2000;286:25–28. [PubMed: 10822144]
- Scott, CR. Sarcosinemia. In: Scriver, CR.; Beaudet, AL.; Valle, D.; Sly, WS., editors. *The Metabolic and Molecular Bases of Inherited Disease*. McGraw Hill, Medical Publishing Division; New York: 2001. p. 2057-2063.
- Sergeeva OA, Chepkova AN, Haas HL. Guanidinoethyl sulphonate is a glycine receptor antagonist in striatum. *Br J Pharmacol* 2002;137:855–860. [PubMed: 12411417]
- Shim SS, Hammonds MD, Kee BS. Potentiation of the NMDA receptor in the treatment of schizophrenia: focused on the glycine site. *Eur Arch Psychiatry Clin Neurosci* 2008;258:16–27. [PubMed: 17901997]
- Smith KE, Borden LA, Hartig PR, Branchek T, Weinshank RL. Cloning and expression of a glycine transporter reveal colocalization with NMDA receptors. *Neuron* 1992;8:927–935. [PubMed: 1534013]
- Thio LL, Clifford DB, Zorumski CF. Characterization of quisqualate receptor desensitization in cultured postnatal rat hippocampal neurons. *J Neurosci* 1991;11:3430–3441. [PubMed: 1719161]
- Thio LL, Shanmugam A, Isenberg K, Yamada K. Benzodiazepines block α_2 -containing inhibitory glycine receptors in embryonic mouse hippocampal neurons. *J Neurophysiol* 2003;90:89–99. [PubMed: 12660352]
- Thio LL, Yamada KA. Differential presynaptic modulation of excitatory and inhibitory autaptic currents in cultured hippocampal neurons. *Brain Res* 2004;1012:22–28. [PubMed: 15158157]
- Tsai G, Lane HY, Yang P, Chong MY, Lange N. Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. *Biol Psychiatry* 2004;55:452–456. [PubMed: 15023571]
- Ueland PM, Middtun O, Windelberg A, Svandal A, Skalevik R, Hustad S. Quantitative profiling of folate and one-carbon metabolism in large-scale epidemiological studies by mass spectrometry. *Clin Chem Lab Med* 2007;45:1737–1745. [PubMed: 17963453]
- Zhang H, Thio LL. Sarcosine, an antischizophrenic agent and glycine transporter I inhibitor, is an inhibitory glycine receptor agonist and NMDA receptor antagonist. 2008 Neuroscience Meeting Planner. 2008 Online Program No. 55.13.
- Zhang HX, Hyrc K, Thio LL. The glycine transport inhibitor sarcosine is an NMDA receptor co-agonist that differs from glycine. *J Physiol* 2009;587:3207–3220. [PubMed: 19433577]
- Zhang HX, Thio LL. Zinc enhances the inhibitory effects of strychnine-sensitive glycine receptors in mouse hippocampal neurons. *J Neurophysiol* 2007;98:3666–3676. [PubMed: 17913992]
- Zhang LH, Gong N, Fei D, Xu L, Xu TL. Glycine uptake regulates hippocampal network activity via glycine receptor-mediated tonic inhibition. *Neuropsychopharmacology* 2008;33:701–711. [PubMed: 17522628]

Figure 1.

Sarcosine evokes a dose-dependent, Cl^- current. (A) Chemical structures for sarcosine and glycine. (B) Currents from one neuron evoked by the indicated sarcosine concentrations. (C) Mean peak current elicited by each sarcosine concentration normalized to the peak current elicited by 10 mM sarcosine in the same cell ($n = 5-11$). Line shows the fit to a logistic equation with an EC_{50} of 3.2 mM and an N of 1.5. Data were obtained at -55 mV and -65 mV and were combined because they were indistinguishable. (D) Ramp IV curves from a neuron before applying sarcosine and during a 3 mM sarcosine application. Using the CsMeSO_3 pipette solution, a neuron was held at 0 mV and subjected to voltage ramps from -100 to +40 mV.

Complete current trace from which the ramp IV curve during a 3 mM sarcosine application was taken (*Inset*).

Figure 2.

Sarcosine evokes a GlyR mediated current. (A) Currents from one neuron evoked by 3 mM sarcosine before applying strychnine, in 30 nM and 1 μ M strychnine, and after washout of strychnine. (B) Mean peak 3 mM sarcosine current in the presence of varying strychnine concentrations normalized to the peak current elicited by 3 mM sarcosine alone ($n = 12$). Line shows the fit to a logistic equation with an IC_{50} of 17 nM and an N of 1.1. (C) Currents from one neuron elicited by 300 μ M glycine, 10 mM sarcosine, and 10 mM sarcosine + 300 μ M glycine. "Sum" trace is the arithmetic sum of the 10 mM sarcosine and 300 μ M glycine currents. (D) Current elicited by 300 μ M glycine before, during a 10 mM sarcosine steady-state current, and after washout of sarcosine. (E) Currents elicited by 10 mM sarcosine before, during a 300 μ M steady-state glycine current, and after washout of glycine. Traces in D and E are at the same scale and are from the same neuron.

Figure 3.

Sarcosine activates GlyRs independently of GlyT1. (A) Currents from one neuron evoked by 3 mM sarcosine, 1 μM NFPS, and 3 mM sarcosine + 1 μM NFPS. The 3 mM sarcosine + 1 μM NFPS current was obtained after applying 1 μM NFPS for ten minutes. (B) Current evoked by 3 mM sarcosine from a neuron in a glial free culture.

Table 1

Sarcosine effects and half-maximal concentrations.

Sarcosine Effect	Concentration for Half-Maximal Effect
GlyT1 Inhibitor	40-150 μM ¹
NMDAR Co-Agonist	26 μM ²
GlyR Agonist	3 mM ³

¹ Smith et al., 1992; Lopez-Corcuera et al., 1998; Herdon et al., 2001; Mallorga et al. 2003.² Zhang et al., 2009.³ Present study.