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ACCELERATED COMMUNICATION

Glycine Site-Directed Agonists Reverse the Actions of Ethanol at the *N*-Methyl-D-aspartate Receptor

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SUMMARY

Ethanol has been shown to inhibit *N*-methyl-D-aspartate (NMDA)-stimulated calcium influx into cerebellar granule cells grown in culture. Because NMDA-mediated responses are modulated by a number of substances, we investigated the effects of several of these agents on ethanol-induced inhibition of calcium flux. Ethanol (50 mM) inhibited NMDA-dependent Ca^{2+} influx by approximately 50%. The percentage of inhibition remained constant with increasing NMDA concentrations (5–250 μM). Increasing Mg^{2+} concentrations in the assay medium inhibited NMDA-stimulated calcium influx but the EC_{50} for Mg^{2+} was unchanged in the presence of ethanol. Glycine at concentrations of 0.3–100 μM potentiated the effects of NMDA. Glycine at concentrations in excess of 10 μM decreased ethanol-mediated inhibition of NMDA-stimulated calcium influx. D-Serine was shown to have effects similar to those of glycine, whereas L-serine was significantly less active in potentiating NMDA-stimulated activity and reversing the ethanol-induced inhibition of calcium influx. *N*-Methylglycine and L-leucine were ineffective in potentiating NMDA actions but high concentrations (1 mM) of *N*-methylglycine attenuated ethanol-induced inhibition, whereas L-leucine (1 mM) had no effect. High concentrations of *N*-methylglycine were shown to reduce glycine-induced enhancement at the NMDA receptor, whereas L-leucine did not affect the glycine response. Glycine did not affect kainate-stimulated calcium influx and did not alter the small amount of inhibition produced by ethanol in the response of the cells to kainate. The results demonstrate that the *in vivo* actions of ethanol on the NMDA systems of brain may be dependent on glycine concentrations at these receptor sites.

Several subtypes of glutamate receptor mediate neurotransmission in the central nervous system. These subtypes of receptor have been designated as NMDA, kainate, quisqualate, and L-2-amino-4-phosphonobutyrate, based on their respective agonist and antagonist specificities (1). The NMDA receptor is unique among the glutamate receptors in that it is part of a receptor-ionophore complex, activation of which allows transmembrane movement of a number of cations including Na^+, K^+, and Ca^{2+}. This system plays a major role in synaptic plasticity (e.g., long term potentiation) and neurotoxicity (e.g., seizures and ischemic damage to neurons) (1, 2). The activity of the NMDA receptor complex is subject to modulation by a variety of agents, including Mg^{2+}, which causes a direct voltage-dependent blockade of the ion channel, Zn^{2+}, which decreases channel function in a voltage-independent manner at a site believed to be peripheral to the channel, and glycine, which acts as an allosteric activator of NMDA receptor-mediated activity (3–5). Glycine modulates NMDA receptor activity through a strychnine-insensitive glycine recognition site, and the occupancy of the glycine binding site has been proposed (6) to be an absolute requirement for activation of this system.

Recent studies in our laboratories (7) and others (8, 9) have demonstrated that ethanol, at concentrations associated with mild to moderate intoxication, selectively inhibits the function of the NMDA receptor, while having little effect on kainate and quisqualate receptor function. Electrophysiologic assessment using patch clamp techniques has demonstrated direct effects of ethanol on the NMDA-gated ion channels rather than actions of ethanol on events secondary to NMDA-induced neuronal depolarization (8). The mechanism by which ethanol produces its effects on the NMDA receptor remains unknown, but our initial work (7) demonstrated that glycine might be involved in this action of ethanol. We undertook the present studies to further examine the interactions between ethanol and glycine, as well as other modulators of NMDA receptor function.

Experimental Procedures

Materials

$^{45}Ca^{2+}$ (18.0–39.5 Ci/mmol) was purchased from New England Nuclear-Dupont (Boston, MA). NMDA, glycine, kainate, strychnine, iso-

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ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; HEPES, *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid.

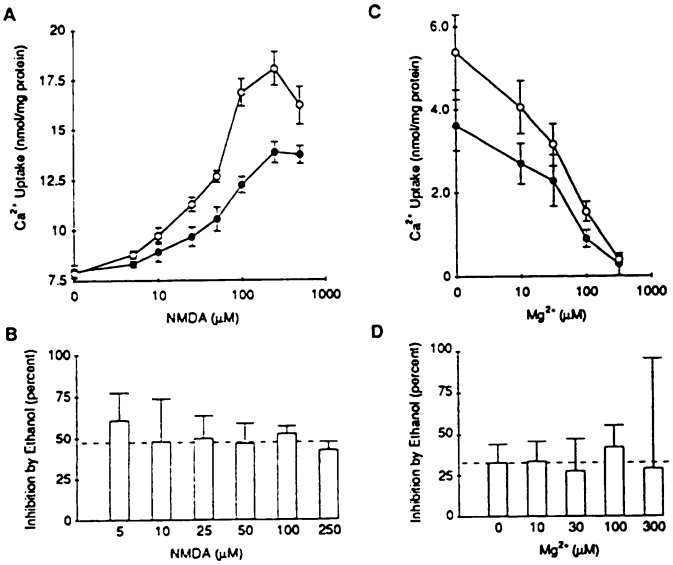

Fig. 1. A and B, Effect of NMDA concentration on ethanol-mediated inhibition of NMDA-stimulated ^{45}Ca uptake. A, Cells were exposed to the concentration of NMDA indicated, in the absence (○) or presence (●) of 50 mM ethanol, and ^{45}Ca uptake was measured as described in Experimental Procedures. B, Inhibition by ethanol is expressed as a percentage of NMDA-stimulated ^{45}Ca uptake at the concentration indicated in the absence of ethanol. C and D, Interaction of Mg^{2+} concentration with ethanol-mediated inhibition of NMDA-stimulated ^{45}Ca uptake. C, Cells were exposed to NMDA (50 μM) and the concentration of Mg^{2+} indicated, in the absence (○) or presence (●) of 25 mM ethanol, and ^{45}Ca uptake was measured. D, Inhibition by ethanol is expressed as a percentage of NMDA-stimulated ^{45}Ca uptake in the presence of the concentration of Mg^{2+} indicated and the absence of ethanol. Values are the mean ± standard error from seven or eight determinations.

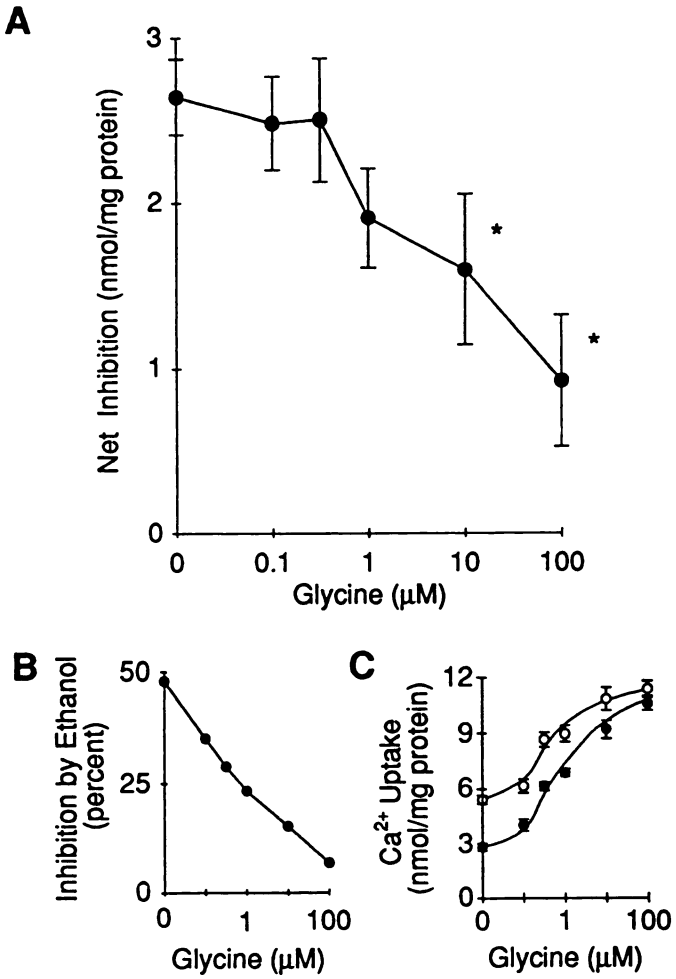

Fig. 2. Effect of glycine on ethanol-mediated inhibition of NMDA-stimulated ^{45}Ca uptake. Cells were exposed to NMDA (50 μM) and the concentration of glycine indicated, in the absence or presence of 50 mM ethanol, and ^{45}Ca uptake was determined. Values are the mean ± standard error of 8–41 determinations. A, Net inhibition is the absolute decrease in NMDA-stimulated ^{45}Ca uptake produced by ethanol at the concentration of glycine indicated. *, Significantly different than the net inhibition produced by ethanol in the absence of glycine at the $p < 0.05$ level, as determined by analysis of variance. B, Inhibition by ethanol is expressed as the percentage of NMDA-stimulated ^{45}Ca uptake in the presence of the concentration of glycine indicated and in the absence of ethanol. C, NMDA-stimulated ^{45}Ca uptake values were obtained in the absence (○) or presence (●) of ethanol (50 mM) and the concentration of glycine indicated.

leucine, and poly-L-lysine were purchased from Sigma Chemical Co. (St. Louis, MO). D- and L-serine and *N*-methylglycine were purchased from Fluka Chemical Corp. (Ronkonkoma, NY). HA-966 was purchased from Tocris Neuramin (Essex, England).

Methods

Cell culture. Primary cultures of cerebellar granule cells were prepared by a modification of the method described by Gallo *et al.* (10) and Novelli *et al.* (11). Briefly, cerebelli were dissected from 8-day-old Sprague-Dawley rats and cells were dissociated and resuspended in basal Eagle's medium containing 10% fetal bovine serum, 25 mM KCl, 2 mM glutamine, and 100 μg/ml gentamycin. After 18 hr, growth medium was aspirated and replaced with chemically defined growth medium (basal Eagle's medium containing 5 μg/ml insulin, 100 μg/ml transferrin, 20 nM progesterone, 100 μM putrescine, 30 nM L-glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin, 5 nM $CuSO_4$, 1.5 μM $FeSO_4$, 1.5 μM $ZnSO_4$, 15 μM hypoxanthine, 1.5 μM thymidine, 50 μM L-alanine, 50 μM L-asparagine, 50 μM glycine, 150 μM L-proline, 50 μM L-serine, and 25 mM KCl) (7). Cytosine arabinofuranoside (10 μM) was included to inhibit replication of nonneuronal cells. On day 4 of culture, approximately 75% of the medium on the cells was aspirated and replaced with fresh defined medium.

$^{45}Ca^{2+}$ uptake. At 8 days *in vitro*, cultures of cerebellar neurons were used to measure $^{45}Ca^{2+}$ uptake. Cultures were washed twice with prewarmed (37°) Locke's saline (5.6 mM KCl, 3.6 mM $NaHCO_3$, 2.3 mM $CaCl_2$, 5 mM HEPES, 154 mM NaCl, and 5.5 mM D-glucose at pH 7.4) with or without 1.0 mM $MgCl_2$ (unless otherwise indicated, experiments measuring NMDA-stimulated $^{45}Ca^{2+}$ uptake used Mg^{2+}-free medium and experiments measuring kainate-stimulated $^{45}Ca^{2+}$ uptake contained Mg^{2+} and 200 μM APV to block any NMDA-stimulated component). Washed cultures were preincubated for 6 min at 37°. Uptake was initiated by aspiration of the preincubation medium and replacement with prewarmed Locke's saline (with or without Mg^{2+}) containing 5 μCi/ml $^{45}Ca^{2+}$ and the combination of drugs indicated in Results. Incubation with isotope was terminated after 60 sec by aspiration of the $^{45}Ca^{2+}$-containing solution and rapid washing with ice-cold Locke's. NaOH (1 N) was added to each culture dish and aliquots were taken to determine $^{45}Ca^{2+}$ uptake (by scintillation counting) and total protein (12). Each NMDA- or kainate-stimulated value reflects subtraction of a simultaneously obtained "basal" value run under identical conditions with the exception of omission of agonist. Statistical analysis of data was performed by use of analysis of variance and *t* test as appropriate. *p* values of <0.05 were taken to indicate statistical significance.

Results

In the absence of added Mg^{2+}, NMDA stimulated Ca^{2+} uptake into cultures of cerebellar neurons in a concentration-depend-

TABLE 1

Effect of glycine analogs on ethanol-mediated inhibition of NMDA-stimulated Ca^{2+} uptake

Cells were exposed to NMDA (50 μM) plus the treatment indicated, in either the absence or presence of ethanol (50 mM), and Ca^{2+} uptake was determined. Values are the mean ± standard error of 8–49 determinations.

	NMDA-stimulated Ca^{2+} uptake		Inhibition
	−Ethanol	+Ethanol	
	nmol/mg of protein		%
No additions	5.4 ± 0.3	2.8 ± 0.2	48.0
+Glycine, 10 μM	10.8 ± 0.6	9.2 ± 0.5	15.1*
+Glycine, 100 μM	11.4 ± 0.4	10.6 ± 0.4	6.8*
+D-Serine, 100 μM	11.9 ± 0.3	9.9 ± 0.5	17.0*
+L-Serine, 100 μM	6.2 ± 0.3	3.9 ± 0.3	38.0
+L-Serine, 1 mM	9.8 ± 0.6	7.7 ± 0.4	21.9*
+*N*-Methylglycine, 100 μM	5.0 ± 0.1	3.4 ± 0.7	32.8
+*N*-Methylglycine, 1 mM	5.3 ± 0.3	4.6 ± 0.3	14.1*
+L-Isoleucine, 1 mM	5.8 ± 0.7	2.7 ± 0.4	52.7
+Spermidine, 1 mM	5.0 ± 0.5	3.0 ± 0.4	40.0

* Significantly different ($p < 0.05$) than the inhibition produced with no additions, as determined by one-way analysis of variance.

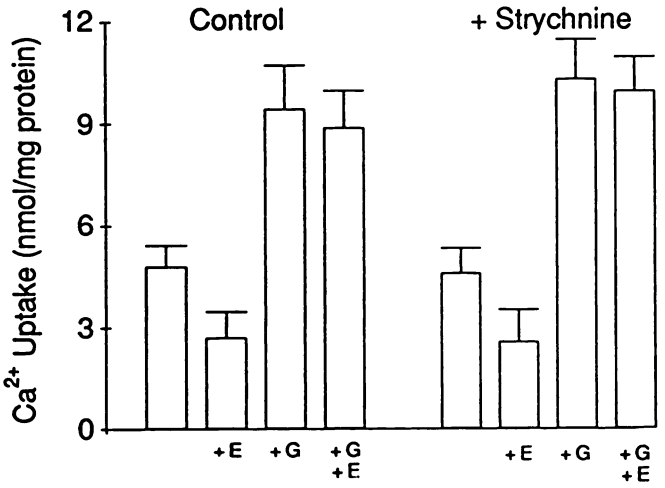

Fig. 3. Cells were exposed to NMDA (50 μM) in the absence or presence of ethanol (*E*) (50 mM). Glycine (*G*) (100 μM) and/or strychnine (1 μM) were also present in the uptake medium, as indicated, and ^{45}Ca uptake was determined. Values are the mean ± standard error of 9–12 determinations.

ent manner (EC_{50} = 50 μM) (Fig. 1A). Ethanol (50 mM) inhibited the NMDA-stimulated uptake of Ca^{2+}. The EC_{50} for NMDA was unchanged by ethanol (Fig. 1A) and the percentage of inhibition produced by ethanol (approximately 48%) remained constant over NMDA concentrations ranging from 5 to 250 μM (Fig. 1B). Addition of Mg^{2+} also inhibited NMDA-stimulated Ca^{2+} uptake into these cultures (IC_{50} = 35 μM) (Fig. 1C). The IC_{50} for Mg^{2+} was unaffected by the simultaneous addition of ethanol (25 mM) (Fig. 1C) and the percentage of inhibition produced by ethanol (approximately 30%) remained constant over the range of Mg^{2+} concentrations tested (Fig. 1D).

Glycine (0.3–100 μM) potentiated NMDA-induced Ca^{2+} uptake into the cultures of cerebellar neurons. The EC_{50} value for glycine under our assay conditions was calculated to be 0.6 μM (Fig. 2). In the absence of added glycine, stimulation of Ca^{2+} uptake by NMDA (50 μM) was completely blocked by inclusion of the glycine antagonist HA-966 (1 mM) in the assay medium. When the inhibitory effect of ethanol was examined in the presence of increasing concentrations of glycine, the inhibition

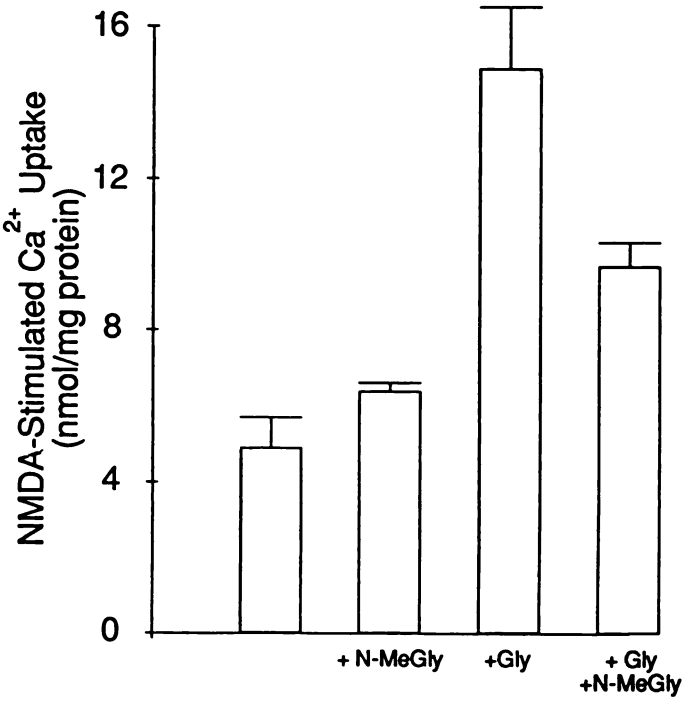

Fig. 4. Cells were exposed to NMDA (50 μM) in either the absence or presence of glycine (10 μM). *N*-Methylglycine (1 mM) was also present where indicated and ^{45}Ca uptake was determined. Values are the mean ± standard error of six to eight determinations.

of Ca^{2+} uptake by ethanol was significantly decreased by concentrations of glycine of 10 μM and greater (Fig. 2). At a concentration of 100 μM, glycine almost completely reversed the actions of ethanol (Fig. 2, Table 1). The enhancement of NMDA-induced Ca^{2+} uptake and the reversal of ethanol-mediated inhibition by glycine (100 μM) were undiminished when assays were carried out in the presence of 1 μM strychnine (Fig. 3). D-Serine reversed the inhibitory effect of ethanol and enhanced the response to NMDA at a concentration similar to that of glycine, whereas L-serine was significantly less active both in enhancing the effect of NMDA and in reversing the inhibition of NMDA-induced Ca^{2+} influx by ethanol (Table 1). On the other hand, *N*-methylglycine (1 mM) produced little or no effect on NMDA-stimulated Ca^{2+} influx but did attenuate the inhibitory effect of ethanol (Table 1). Although some diminution of the inhibition produced by ethanol was observed with 100 μM *N*-methylglycine, this effect did not reach statistical significance. Evidence for the interaction of *N*-methylglycine with the glycine binding site on the NMDA receptor complex was obtained by examination of the effects of glycine in the presence of 1 mM *N*-methylglycine (Fig. 4). *N*-Methylglycine diminished the stimulatory effect of glycine. L-Isoleucine (1 mM) was ineffective in potentiating NMDA-induced Ca^{2+} influx and did not diminish the action of ethanol (Table 1). Also, L-isoleucine (1 mM) had no effect on the ability of 10 μM glycine to enhance 50 μM NMDA-stimulated Ca^{2+} uptake (data not shown). Spermidine (1 mM) produced no significant effect either on NMDA-induced Ca^{2+} uptake or on the inhibition of NMDA-stimulated Ca^{2+} uptake by ethanol (Table 1).

Kainate-stimulated Ca^{2+} uptake in cerebellar granule cells was inhibited approximately 20% by 50 mM ethanol (kainate at 100 μM: 5.5 ± 0.3; kainate plus ethanol: 4.3 ± 0.3 nmol/mg of protein; three or four experiments/group). Addition of 100

μM glycine did not potentiate the effects of kainate and did not alter the inhibitory actions of ethanol (kainate at 100 μM plus glycine: 5.3 ± 0.2; kainate, glycine, and ethanol: 4.2 ± 0.2 nmol/mg of protein; four experiments/group).

Discussion

Ethanol has been demonstrated to be a potent and selective inhibitor of NMDA receptor-mediated transmembrane events (7, 8) and neurotransmission (13). In the current study, we have demonstrated that addition of high concentrations of glycine (10 μM and higher) could reverse ethanol-induced inhibition of NMDA-stimulated Ca^{2+} uptake. The reversal of ethanol-mediated inhibition at the NMDA receptor by increasing concentrations of glycine also has been postulated by Woodward and Gonzales (14) in their studies of NMDA-stimulated dopamine release from slices of rat striatum.

The concentrations of glycine necessary for reversal of the inhibitory effects of ethanol in the cerebellar granule cell cultures were significantly higher than the EC_{50} values for glycine-induced potentiation of NMDA-stimulated activity measured electrophysiologically (300 nM) (15), in binding experiments (300 nM) (16), or in the Ca^{2+} uptake experiments described here (600 nM). The discrepancy between concentrations associated with reversal of the effects of ethanol and potentiation of NMDA-stimulated responses might suggest separate sites for these effects. However, several observations argue against the reversal of the effects of ethanol by glycine at another known or nonspecific site. First, it is unlikely that the strychnine-sensitive glycine site was involved, because the reversal of ethanol-induced inhibition was unaltered by 1 μM strychnine. Second, it is unlikely that glycine caused reversal of ethanol-mediated inhibition by acting at a nonspecific site to produce a generalized increase in Ca^{2+} uptake, because all NMDA-stimulated values obtained in the presence of glycine have had values obtained in the presence of glycine but the absence of agonist (basal values) subtracted (see Experimental Procedures). Furthermore, it is unlikely that glycine caused reversal of ethanol-mediated inhibition by acting at a nonspecific site to produce a generalized blockade of the effects of ethanol, because glycine had no effect on the small amount of inhibition of kainate-stimulated Ca^{2+} uptake produced by ethanol.

The most compelling evidence for the site of action of glycine is the finding that the potency of a variety of amino acids in reversing ethanol-mediated inhibition parallels the structural requirements of the NMDA receptor-associated glycine binding site, as described by McBain *et al.* (15). D-Serine was similar to glycine in its action and was more potent than its L-isomer, in terms of both stimulating NMDA-dependent Ca^{2+} uptake and reversing ethanol-mediated inhibition. Isoleucine (1 mM), most likely because of the bulky substitution on the α-carbon (15), was inactive in stimulating NMDA action and in reversing ethanol-induced inhibition. Isoleucine was also without effect when added to assay systems where glycine was present together with NMDA, indicating that this amino acid probably does not compete for occupancy of the glycine binding site. Substitution of an *N*-methyl group on the amino group of glycine has been found to significantly attenuate the ability of glycine to potentiate NMDA actions (15). Our studies (Table 1, Fig. 4) also demonstrate the lack of potency of *N*-methylglycine in increasing NMDA-stimulated Ca^{2+} uptake. However, 1 mM *N*-methylglycine significantly reduced the inhibition of NMDA-stimulated Ca^{2+} uptake by 50 mM ethanol. Unlike isoleucine, the addition of *N*-methylglycine to cultures containing glycine and NMDA reduced the stimulation of NMDA actions produced by glycine, indicating that high concentrations of *N*-methylglycine (1 mM) may compete for the glycine binding site.

At present it is unclear how glycine mediates the reversal of ethanol-induced inhibition of NMDA receptor function. Direct measurements of [^{3}H]glycine binding to a site on the NMDA receptor[1] demonstrate that ethanol (up to 200 mM) has no significant effect on equilibrium binding constants for glycine. Recent reports on the mechanism by which glycine potentiates NMDA-stimulated activity indicate that glycine may increase the frequency with which the NMDA receptor ionophore opens or may increase the rate of recovery of the NMDA receptor from the desensitized state (5, 17). Therefore, one could speculate that ethanol-induced inhibition of NMDA-stimulated activity results from an ethanol-induced decrease in the frequency of channel opening or increase in the rate of desensitization of the receptor in the presence of NMDA receptor agonists, and glycine could counteract these effects of ethanol. This speculation is consistent with the data in the current and previous reports on the effects of ethanol at this receptor system and can explain the reversal of the actions of ethanol by glycine and closely related amino acids.

The effect of glycine on ethanol-induced inhibition of NMDA-stimulated activity raises questions as to whether the actions of ethanol witnessed *in vitro* may be responsible for any of the *in vivo* effects of ethanol. As already mentioned, the EC_{50} value for glycine-mediated potentiation of NMDA action in isolated washed membrane preparations is below 1 μM, whereas glycine concentrations in the cerebrospinal fluid are approximately 7 μM (18). It is of interest, therefore, that significant reversal of ethanol-mediated inhibition of NMDA-stimulated Ca^{2+} uptake in the cerebellar granule cells required concentrations of glycine of 10 μM or more. Glycine-mediated changes in other NMDA-induced actions (e.g., cytotoxicity) on cultured cells also required high concentrations (20–30 μM) of glycine (19, 20). Uptake and metabolic barriers may diminish glycine levels at the NMDA receptor below saturating concentrations *in vivo* and would allow ethanol to inhibit the function of this glutamatergic receptor system. Drugs that mimic the actions of glycine may be useful for reversing certain of the acute effects of ethanol intoxication.

[1] Snell L., Tabakoff, B. and Hoffman, P. L., Manuscript in preparation.

References

1. Foster, A., and G. E. Fagg. Acidic amino acid binding sites in mammalian neuronal membranes; their characteristics and relationship to synaptic receptors. *Brain Res.* **319**:103–164 (1984).
2. Mayer, M. L., and G. L. Westbrook. The physiology of excitatory amino acids in the vertebrate central nervous system. *Prog. Neurobiol.* **28**:197–276 (1987).
3. Nowak, L. P., P. Bregestovski, P. Ascher, A. Herbet, and A. Prochiantz. Magnesium gates glutamate-activated channels in mouse central neurones. *Nature (Lond.)* **307**:462–465 (1984).
4. Mayer, M. L., G. L. Westbrook, and L. Vyklicky. Sites of antagonist action on *N*-methyl-D-aspartic acid receptors studied using fluctuation analysis and a rapid perfusion technique. *J. Neurophysiol.* **60**:645–663 (1988).
5. Johnson, J. W., and P. Ascher. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature (Lond.)* **325**:529–531 (1987).
6. Kleckner, N. W., and R. Dingledine. Requirement for glycine in activation of NMDA receptors expressed in *Xenopus* oocytes. *Science (Washington, D. C.)* **241**:835–837.
7. Hoffman, P. L., C. S. Rabe, F. Moses, and B. Tabakoff. *N*-Methyl-D-aspartate

receptors and ethanol: inhibition of calcium flux and cyclic GMP production. *J. Neurochem.* **52**:1937–1940 (1989).
8. Lovinger, D. M., G. White, and F. F. Weight. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science (Washington, D. C.)* **243**:1721–1724 (1989).
9. Lima-Landman, M. T. R., and E. X. Albuquerque. Ethanol potentiates and blocks NMDA-activated single-channel currents in rat hippocampal pyramidal cells. *FEBS Lett.* **247**:61–67 (1989).
10. Gallo, V., M. T. Ciotti, A. Coletti, F. Aloisi, and G. Levi. Selective release of glutamate from cerebellar granule cells differentiating in culture. *Proc. Natl. Acad. Sci. USA* **79**:7919–7923 (1982).
11. Novelli, A., F. Nicoletti, J. T. Wroblewski, H. Alho, E. Costa, and A. Guidotti. Excitatory amino acid receptors coupled with guanylate cyclase in primary cultures of cerebellar granule cells. *J. Neurosci.* **7**:40–47 (1987).
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275 (1951).
13. Göthert, M., and K. Fink. Inhibition of *N*-methyl-D-aspartate (NMDA)- and L-glutamate-induced noradrenaline and acetylcholine release in the rat brain by ethanol. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **340**:516–521 (1989).
14. Woodward, J. J., and R. A. Gonzales. Ethanol inhibition of *N*-methyl-D-aspartate-stimulated endogenous dopamine release from rat striatal slices: reversal by glycine. *J. Neurochem.* **54**:712–715 (1990).
15. McBain, C. J., N. W. Kleckner, S. Wyrick, and R. Dingledine. Structural requirements for activation of the glycine coagonist site of *N*-methyl-D-aspartate receptors expressed in *Xenopus* oocytes. *Mol. Pharmacol.* **36**:556–565 (1989).
16. Bonhaus, D. W., G.-C. Yeh, L. Skaryak, and J. O. McNamara. Glycine regulation of the *N*-methyl-D-aspartate receptor-gated ion channel in hippocampal membranes. *Mol. Pharmacol.* **36**:273–279 (1989).
17. Mayer, M. L., L. Vyklicky, Jr., and J. Clements. Regulation of NMDA receptor desensitization in mouse hippocampal neurons by glycine. *Nature (Lond.)* **338**:425–427 (1989).
18. Clarke, D. D., A. L. Lajtha, and H. S. Maker. Intermediary metabolism, in *Basic Neurochemistry* (G. Siegel, B. Agranoff, R. W. Albers, and P. Molinoff, eds.), Ed. 4. Raven Press, New York 541–564 (1989).
19. McNamara, D., and R. Dingledine. Requirement for glycine in NMDA-induced excitotoxicity in rat cortical cell culture. *Soc. Neurosci Abstr.* **195(11)**:481.
20. Uckele, J. E., J. W. McDonald, M. V. Johnston, and F. S. Silverstein. Effect of glycine and glycine receptor antagonists on NMDA-induced brain injury. *Neurosci. Lett.* **107**:279–283 (1989).

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