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# Glycine Enhances the Central Depressant Properties of Ethanol in Mice

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WILLIAMS, K. L., A. P. FERKO, E. J. BARBIERI AND G. J. DiGREGORIO. *Glycine enhances the central depressant properties of ethanol in mice.* PHARMACOL BIOCHEM BEHAV 50(2) 199–205, 1995. – The interaction between ethanol and glycine in the central nervous system was investigated in male Swiss-Webster mice. The loss of the righting reflex (LORR) was used as a measure of central nervous system depression. Mice were injected with ethanol (4.0 g/kg, IP), causing an ethanol-induced LORR. Immediately after the animals regained the righting reflex from ethanol administration, they received an intracerebroventricular (ICV) injection of saline or glycine (1, 15, 25, or 50 μmol/kg) in a volume of 5 μl. Upon ICV injection of glycine, the mice lost the righting reflex once again. This effect of glycine in the presence of ethanol occurred rapidly and in a dose-dependent manner. Glycine induced a return to the LORR of 12.6 ± 0.7, 24.5 ± 1.3, 32.8 ± 2.0, and 46.8 ± 4.5 min when doses of 1, 15, 25, and 50 μmol/kg, respectively, were injected. D-Serine (15, 25, or 50 μmol/kg), an amino acid precursor of glycine, was injected (ICV) after the animals regained the righting reflex following ethanol injection (IP). Serine caused a return to the LORR of 0.5 ± 0.5, 6.0 ± 1.0, and 6.5 ± 0.9 min when doses of 15, 25, and 50 μmol/kg, respectively, were injected. Strychnine was used to attenuate the ability of glycine and serine to cause a return to the LORR in the presence of ethanol. Strychnine, a competitive antagonist of glycine, significantly reduced the ability of glycine and serine to enhance the depressant action of ethanol. When glycine (50 μmol/kg), serine (50 μmol/kg), and stychnine (300 nmol/kg) were administered in the absence of ethanol, no significant return to the LORR was observed. Bicuculline, a GABA antagonist, was administered at a dose of 10 nmol/kg in combination with 25 μmol/kg of glycine. No significant reduction in glycine-induced return to the LORR was observed. This suggests that glycine is augmenting the effect of ethanol without stimulation of the GABAergic system. The results of this study indicate that glycine, an inhibitory neurotransmitter, can augment the central depressant properties of ethanol by acting on the strychnine-sensitive glycine receptor site.

Ethanol Glycine Strychnine Serine CNS depression Loss of the righting reflex Sleep time Bicuculline

ETHANOL has long been known to produce changes in behavioral and cognitive function by its depressant effect on the central nervous system. The exact mechanisms by which ethanol achieves this effect have yet to be elucidated. Ethanol can alter the fluidity of membranes (17) and affect ion channels and neurotransmitter release. Recent evidence suggests that the acute and chronic effects of ethanol result from its ability to specifically and selectively affect ionic flux through membrane channels (7,33). The focus of many recent studies has been on the amino acid neurotransmitter, gamma-aminobutyric acid (GABA) and on the *N*-methyl-D-aspartate (NMDA) receptor complex. A large body of evidence suggests that ethanol enhancement of GABA, a major inhibitor neurotransmitter in the mammalian brain, may be responsible for the central depressant effects of ethanol (1,6). The interaction of ethanol with the excitatory neurotransmitter glutamate at the NMDA receptor in the CNS is less clear. The effect of NMDA on its receptor complex, which is one of the subgroups of receptors for glutamate, has been shown to be inhibited by ehtanol (23,30). Furthermore, NMDA has been shown to be capable of releasing GABA (9,20). The focus of the present work continues to consider the possible involvement of another amino acid neurotransmitter, glycine, in the actions of ethanol.

Glycine is a inhibitory neurotransmitter in the spinal cord and brain that regulates motor and sensory functions (18) by acting at a postsynaptic membrane receptor (4). Strychnine is a competitive antagonist of the postsynaptic glycine receptor

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but does not effect GABA-mediated chloride fluxes (11,16). The use of strychnine has allowed the categorization of two glycine receptor sites; the strychnine-sensitive site, which is associated with regulation of chloride flux on postsynaptic membranes (10,11), and the strychnine-insensitive site, which is located on the NMDA receptor, where it appears to act as a modulator (28). Recent in vitro studies show that ethanol potentiates chloride flux through the glycine receptor ionophore (6), which is blocked by strychnine. Current studies (26,30) seem to suggest that ethanol does not affect the glycine-specific chloride ionophore. This current evidence and controversy prompted the investigation of the interaction of glycine with ethanol in an in vivo model.

In this investigation, glycine was administered intracerebroventricularly (ICV) in the presence of ethanol. The hypothesis of this investigation was that glycine would enhance the central depressant effects of ethanol. Strychnine, a glycine receptor antagonist, was injected in combination with glycine to determine the site and nature of the activity of glycine. Bicuculline, a GABA receptor antagonist, was injected in combination with glycine to help further delineate the nature of the effect of glycine. Serine, a precursor to glycine, was also administered to determine its effects on the CNS in the presence of ethanol. The loss of the righting reflex (LORR) was used to measure the degree of CNS depression produced by ethanol.

## METHOD

Animals were male Swiss-Webster mice obtained from Taconic Laboratory (New York, NY) weighing 27-35 g. All of the mice were housed for 1 week prior to experimentation. This environment consisted of a light cycle from 0600 to 1800 h, a temperature of 22 ± 1°C, and free access to water and Purina Laboratory Chow (Ralston Purina Co., St. Louis, MO). The ethanol solution for IP injection was prepared from 95% (v/v) ethanol and diluted to 20% (w/v) with saline (0.9% NaCl). Glycine, serine, strychine, and bicuculline methiodide were obtained from Sigma Chemical Co. (St. Louis, MO). Glycine was prepared in the hydrochloride form and the D-isomer of serine was used. Glycine (1, 15, 25, and 50 μmol/kg), serine (15, 25, and 50 μmol/kg), strychine (50, 100, and 300 nmol/kg), and bicuculline (10 nmol/kg) were prepared with saline (0.9% NaCl) and adjusted to pH 7.0 with NaOH solution (12,13). These solutions were prepared prior to every experimental trial. All other chemicals were obtained from commercial sources and were of analytical grade.

### *Acute Ethanol Administration and Loss of the Righting Reflex (LORR) as an Index of CNS Depression*

The mice received an IP injection of ethanol (4.0 g/kg) that induced a LORR. The concentration of a 20% solution of ethanol minimized irritation to the tissue (35). The environment and conditions under which the experiments were performed were kept as constant as possible; the experiments were initiated between 0900 and 1000 h, external noise was kept to a minimum, and animals were kept from contact with each other during the LORR time period of the experiment.

The duration of the LORR was used as an index of central nervous system depression. The time of injection to the ethanol-induced LORR was recorded in seconds and referred to as time to the *onset of the LORR*. The interval between the initial LORR and the subsequent gain of the righting reflex was recorded and referred to as the *ethanol LORR*. A second period of the LORR was experienced by animals after ICV injection of a drug. This second period was measured from the time of ICV injection to the subsequent regain of the righting reflex and is referred to as the *return to the LORR*. The end of LORR for these experiments refers to the ability of the mouse to right itself by rolling onto its feet three times within 15 s when placed on its back.

### *ICV Drug Administration*

The following procedure was used for ICV injection. Twenty minutes after the animal lost the righting reflex following IP ethanol administration, a sagittal incision was made on the dorsal aspect of the head exposing the skull sutures. A hole 3 mm deep was made 2 mm caudal to the bregma suture and 2 mm lateral to the sagittal suture using a 26-ga needle. The previous step was performed on an ethanol-anesthetized mouse to gain access to the ventricle of the brain for later ICV drug administration (preparatory injection). Immediately after the animals regained the righting reflex following the IP injection of ethanol, they were given an ICV injection of saline or drug solution (total volume of 5 μl). This ICV injection was administered over a period of 10 s. Upon ICV injection of saline or drug solution, a second LORR was recorded (return to LORR). When these animals regained the righting reflex, following ICV drug injection, a 20-μl blood sample was obtained from the orbital sinus to determine the blood ethanol concentration. The correct position of the ICV drug administration was verified upon autopsy by ICV injection of trypan blue and subsequent dissection.

### *LORR Experiments With Ethanol (IP) and Glycine (ICV) and the Antagonism of Glycine by Strychnine*

The purpose of these experiments was to determine if ICV injection of glycine could cause a return to the LORR, when administered after an ethanol-induced LORR. The procedure used was as previously described (12,15). Immediately after the mice regained the righting reflex following ethanol injection (IP), the animals were injected ICV with saline or glycine (1, 15, 25, or 50 μmol/kg) in a volume of 5 μl.

The next experiment with strychnine and glycine involved ICV administration of saline or several doses (50, 100, or 300 nmol/kg) of strychnine together with glycine (15 μmol/kg). The drugs were administered in a total volume of 5 μl immediately after the animals regained righting reflex following ethanol injection.

The final experiment with strychnine was to determine if strychnine could augment ethanol's depressant effects by itself. This experiment involved ICV administration of 5 μl of strychnine (300 nmol/kg) by itself, after the animals regained the righting reflex.

### *LORR Experiments With Ethanol (IP) and D-Serine (ICV)*

The purpose of these experiments was to ascertain if ICV injection of serine could cause a return to the LORR after the animals regained the righting reflex following the ethanol injection (IP). Immediately after the mice regained the righting reflex after ethanol, the animals were injected ICV with saline or serine (15, 25, or 50 μmol/kg) in a volume of 5 μl.

The effect of strychnine on the serine return to the LORR was investigated as well. Strychnine (100 nmol/kg) was injected (ICV) with 25 μmol/kg of serine in a total volume of 5 μl, immediately after the animals regained the righting reflex from ethanol injection (IP).

*Effect of Bicuculline on the Glycine Return to the LORR*

These experiments were done to determine if bicuculline, a GABA receptor antagonist, could reduce the effect of glycine to cause a return to the LORR. Immediately after regaining the righting reflex following ethanol injection (IP), the animals received an ICV injection of (a) saline, (b) 25 μmol/kg of glycine, (c) 25 μmol/kg of glycine and 10 nmol/kg of bicuculline, or (d) 10 nmol/kg of bicuculline alone.

*LORR Experiments With Glycine, D-Serine, Strychnine, and Bicuculline in the Absence of Ethanol*

In these experiment, saline, glycine, serine, strychnine, or bicuculline was administered ICV in the absence of ethanol to note if any of the compounds could cause a LORR by itself. Mice were injected with saline (0.02 ml/g, IP) to maintain a volume equal to that given to ethanol-treated animals. Twenty minutes later the animals were lightly anesthetized with methoxyflurane. At this time, an ICV preparatory procedure was performed as described previously. The average time elapsed between ethanol administration and drug administration in the ethanol-injected animals in this study was 50 min. Utilizing this fact to keep the parameters as similar as possible, 50 min after IP injection of saline, the mice were heavily sedated with methoxyflurane without causing a LORR and injected with 5 μl of (a) saline, (b) 50 μmol/kg of glycine, (c) 50 μmol/kg of serine, or (d) 300 μmol/kg of strychnine. Observations of the animals were made for 2 h following drug administration.

*Blood Ethanol Analysis*

Blood was taken from the orbital sinus of the mouse, collected in a 20-μl Sahli pipette, and assayed according to Lundquist (24). The blood was deproteinized by its addition to 200 μl of 3.4% perchloric acid. This blood mixture was centrifuged in a Sorvall RC-2 at 4300 × *g* for 5 min and the supernatant was removed for analysis. The filtrate (50 μl) was added to tubes containing 3 ml of prepared reaction mixture containing buffer solution, NAD solution, and alcohol dehydrogenase suspension. The optical density was read after a 60-min incubation period at room temperature on a Milton Roy CoSpectronic 601 spectrophotometer at 340 nm.

*Statistical Analysis*

Statistically significant differences were determined by analysis of variance (ANOVA). All multiple comparisons with a control and comparison among experimental groups were done by ANOVA followed by Scheffe's test. Data are expressed as mean ± SE in the tables.

## RESULTS

*Effect of Glycine on the LORR in the Presence of Ethanol and the Antagonism of Glycine's Effect by Strychnine*

All of the animals experienced a LORR after ethanol administration (Tables 1–4). The onset of the LORR was approximately 90 s; ethanol LORR lasted for approximately 45–50 min. There were no statistically significant differences in onset to LORR or in the ethanol LORR among any of the experimental groups. Glycine caused an augmentation of the central depressant properties of ethanol in mice. This glycine return to the LORR occurred immediately after ICV injection of the compound and in a dose-dependent manner (Table 1). The blood ethanol levels taken after the mice regained the righting reflex following glycine administration (ICV) demonstrate an inverse relationship between blood ethanol levels and the doses of glycine.

The data in Table 2 show that when different doses of strychnine were administered (50, 100, or 300 nmol/kg) together with glycine (15 μmol/kg), strychnine inhibited the effects of glycine in a dose-dependent manner. The fact that the 50-nmol/kg dose of strychnine showed no significant reduction in the glycine return to the LORR when compared to the glycine dose alone indicated that the 50-nmol/kg strychnine dose was at or near the threshold dose. The 300-nmol/kg dose of strychnine completely antagonized the effect of glycine, indicating that the ceiling effect had been reached. Most of the animals experienced excessive scratching motions to the head and abdominal flexion upon ICV administration of strychnine. In addition, strychnine (300 nmol/kg, ICV) was administered by itself to six animals after the ethanol-induced LORR, to determine if strychnine could cause a LORR. The results showed that no LORR was observed for any of the animals (data not shown). Three animals displayed minor convulsions for 10 s after injection, but were otherwise normal.

TABLE 1

THE EFFECT OF GLYCINE (GLY) ON THE RETURN TO THE LOSS OF THE RIGHTING REFLEX (LORR) WHEN ADMINISTERED IMMEDIATELY AFTER REGAINING THE RIGHTING REFLEX FROM ETHANOL (ETOH) INJECTION

| Group | *N* | Onset to LORR (s) | ETOH-LORR (min) | GLY Return to LORR* (min) | Blood ETOH (mg/ml) |
|---|---|---|---|---|---|
| ETOH† + Saline | 8 | 93.9 ± 6.8 | 45.4 ± 3.2 | 0.1 ± 0.1 | 3.60 ± 0.06 |
| ETOH + GLY (1) | 8 | 86.1 ± 3.3 | 49.3 ± 3.2 | 12.6 ± 0.7‡ | 3.38 ± 0.05 |
| ETOH + GLY (15) | 8 | 88.8 ± 6.1 | 47.4 ± 3.1 | 24.5 ± 1.3‡,§ | 3.25 ± 0.05‡ |
| ETOH + GLY (25) | 8 | 83.0 ± 5.4 | 48.9 ± 2.0 | 32.8 ± 2.0‡,§ | 3.02 ± 0.04‡,§ |
| ETOH + GLY (50) | 8 | 94.4 ± 2.7 | 47.3 ± 2.8 | 46.8 ± 4.5‡,§,¶,# | 2.84 ± 0.08‡,§,¶ |

*Glycine injected (μmol/kg, ICV) immediately after regaining the righting reflex following ETOH injection.
†ETOH was given at 4.0 g/kg IP.
‡Significantly different from controls ($p < 0.05$).
§Significantly different from GLY (1) group ($p < 0.05$).
¶Significantly different from GLY (15) group ($p < 0.01$).
#Significantly different from GLY (25) group ($p < 0.01$).

TABLE 2

STRYCHNINE (STR) ANTAGONIZES THE DURATION OF THE RETURN TO THE LORR INDUCED BY GLYCINE IN THE PRESENCE OF ETHANOL (ETOH)

| Group | N | Onset to LORR (s) | ETOH-LORR (min) | GLY Return to LORR* (min) | Blood ETOH (mg/ml) |
|---|---|---|---|---|---|
| ETOH† + Saline | 8 | 92.5 ± 5.3 | 43.7 ± 2.6 | 0.1 ± 0.1 | 3.58 ± 0.06 |
| ETOH + GLY (15) | 8 | 88.8 ± 6.1 | 47.4 ± 3.1 | 24.5 ± 1.3‡ | 3.25 ± 0.05‡ |
| ETOH + GLY (15) + STR (50) | 8 | 86.5 ± 4.5 | 52.0 ± 2.7 | 23.6 ± 1.2‡ | 3.06 ± 0.04‡ |
| ETOH + GLY (15) + STR (100) | 8 | 96.3 ± 6.2 | 44.6 ± 2.1 | 14.7 ± 15‡,§,¶ | 3.43 ± 0.05¶ |
| ETOH + GLY (15) + STR (300) | 9 | 84.7 ± 3.3 | 45.9 ± 0.9 | 0.1 ± 0.1§,¶,# | 3.43 ± 0.02¶ |

*Glycine (μmol/kg), strychnine (nmol/kg), or both were injected (ICV) immediately after regaining the righting reflex following ETOH administration.
†ETOH was given at 4.0 g/kg, IP.
‡Significantly different from controls ($p < 0.01$).
§Significantly different from GLY (15) group ($p < 0.01$).
¶Significantly different from GLY (15) + STR (50) group ($p < 0.01$).
#Significantly different from GLY (15) + STR (100) group ($p < 0.1$).

*Effect of* D-*Serine on LORR in the Presence of Ethanol*

Table 3 shows that serine can enhance the central depressant properties of ethanol as measured by the serine return to the LORR. The 25- and 50-μmol/kg doses of serine caused a small, but statistically significant, return to the LORR when compared to the control but did not differ significantly from one another, apparently demonstrating a ceiling effect. The 15-μmol/kg dose of serine caused no significant effect when compared to the control, indicating that it was a subthreshold dose.

Doses of glycine and serine were compared to one another to determine their relative ability to cause a return to the LORR when administered in the presence of ethanol. When identical doses of glycine and serine (15, 25, and 50 μmol/kg) were compared to one another, serine exhibited significantly ($p < 0.01$) less ability to cause a return to the LORR than glycine (Tables 1 and 3).

In an effort to further understand the site of action of serine, the glycine receptor antagonist strychnine was administered together with serine. The results displayed in Table 3 show a significant reduction in serine return to the LORR when compared to 25 μmol/kg of serine by itself. These findings support the idea that serine may act as a weak glycine receptor agonist.

*Effect of Bicuculline on Glycine Return to LORR*

The data in Table 4 show the effect of bicuculline, a $GABA_A$ receptor antagonist, on the glycine return to the LORR. There was no significant difference in the glycine return to LORR between those animals administered glycine (25 μmol/kg) only and those animals given both glycine (25 μmol/kg) and bicuculline (10 nmol/kg). These results indicate that glycine is not interacting with $GABA_A$ receptors to augment the depressant effects of ethanol. When bicuculline (10 nmol/kg) was administered in the absence of glycine, no significant LORR was observed when compared to the saline controls. However, most animals exhibited an excitatory effect ranging from mild running activity to tonic-clonic convulsions.

*Effect of Glycine,* D-*Serine, Strychnine, and Bicuculline in the Absence of Ethanol*

In the next set of experiments, saline, glycine, serine, strychnine, or bicuculline was administered in the absence of

TABLE 3

INTERACTION BETWEEN ETHANOL (ETOH) AND SERINE (SER) ON THE DURATION OF THE RETURN TO THE LORR AND THE ANTAGONISM WITH STRYCHNINE (STR)

| Group | N | Onset to LORR (s) | ETOH-LORR (min) | Serine Return to LORR* (min) | Blood ETOH (mg/ml) |
|---|---|---|---|---|---|
| ETOH† + Saline | 8 | 93.1 ± 4.1 | 40.8 ± 2.2 | 0.1 ± 0.1 | 3.57 ± 0.06 |
| ETOH + SER (15) | 6 | 90.2 ± 4.1 | 45.2 ± 1.7 | 0.5 ± 0.4 | 3.48 ± 1.0 |
| ETOH + SER (25) | 8 | 84.4 ± 3.1 | 46.0 ± 2.6 | 6.0 ± 1.0§,¶,** | 3.30 ± 0.05# |
| ETOH + SER (50) | 8 | 82.9 ± 3.7 | 50.9 ± 2.9 | 6.5 ± 0.9§,¶,** | 3.38 ± 0.07 |
| ETOH + SER (25) + STR (100)‡ | 8 | 86.0 ± 1.4 | 48.2 ± 3.0 | 0.5 ± 0.3 | 3.63 ± 0.08 |

*Serine injected (μmol/kg, ICV) immediately after regaining the righting reflex from ETOH injection.
†ETOH was given at 4.0 g/kg, IP.
‡Serine (μmol/kg) and strychnine (nmol/kg) injected together ICV.
§Significantly different from controls ($p < 0.01$).
¶Significantly different from SER (15) group ($p < 0.01$).
#Significantly different from SER (25) + STR (100) group ($p < 0.05$).
**Significantly different from SER (25) + STR (100) group ($p < 0.01$).

TABLE 4

THE EFFECT OF BICUCULLINE (BIC) ON THE GLYCINE (GLY) RETURN TO LOSS OF THE RIGHTING (LORR) REFLEX WHEN ADMINISTERED TOGETHER IMMEDIATELY AFTER REGAINING THE RIGHTING REFLEX FOLLOWING ETHANOL (ETOH) INJECTION

| Group | N | Onset to LORR (s) | ETOH/LORR (min) | GLY Return to LORR* (min) | Blood ETOH (mg/ml) |
|---|---|---|---|---|---|
| ETOH† + Saline | 7 | 96.6 ± 7.5 | 48.5 ± 3.3 | 0.2 ± 0.1 | 3.46 ± 0.05 |
| ETOH + GLY (25) | 8 | 82.8 ± 3.5 | 48.6 ± 2.0 | 27.9 ± 2.4‡ | 3.11 ± 0.03‡ |
| ETOH + GLY (25) + BIC (10) | 8 | 90.5 ± 4.1 | 50.9 ± 2.8 | 24.8 ± 3.2‡ | 3.22 ± 0.03 |
| ETOH + BIC (10) | 8 | 89.0 ± 2.2 | 50.1 ± 1.3 | 0.4 ± 0.2§,¶ | 3.57 ± 0.12§,¶ |

*Glycine (μmol/kg), bicuculline (nmol/kg), or both were injected (ICV) immediately after regaining the righting reflex following ethanol injection.

†ETOH was given at 4.0 g/kg, IP

‡Significantly different from controls ($p < 0.01$).

§Significantly different from GLY (25) group ($p < 0.01$).

¶Significantly different GLY (25) + BIC (10) group ($p < 0.01$).

ethanol to determine if any of the agents could induce a LORR by itself. The mice were injected with saline (IP) and 50 min later were given an ICV injection of glycine, serine, strychnine, or bicuculline. The saline controls ($n = 5$) did not lose the righting reflex and were normal during the 2-h observation period. The glycine group ($n = 6$) received a 50-μmol/kg dose of glycine and lost the righting reflex for 2.9 ± 0.7 min. All of the animals in this group experienced mild convulsions, two of the animals died, and 50% of the animals displayed a small degree of cyanosis that lasted for the duration of the LORR. During the 2-h observation period all of the animals' behavior was normal after regaining the righting reflex. The serine group ($n = 5$) received a 50-μmol/kg dose and lost the righting reflex for 0.1 ± 0.1 min. They were normal upon drug administration and during the 2-h observational period after drug administration. The strychnine group ($n = 3$) received a 300-nmol/kg dose (the highest doses used in the study) and two animals died within a minute. They displayed a curved posture, tremors, and hyperexcitability before death. The bicuculline group ($n = 2$) received a 10-nmol/kg dose. One of the animals ran wildly for 8 min and the other went into convulsions for 2 min, after which their behavior was similar to the control group.

## DISCUSSION

An accurate measure of the depressant actions of ethanol on the CNS has proven to be the LORR (32). The present study showed that ICV administration of glycine in this animal model caused a dose-dependent effect in returning the animals to a second LORR after an ethanol-induced LORR (Table 1). These effects were found to be antagonized by strychnine in a dose-dependent manner, weakly mimicked by serine, and unaffected by the $GABA_A$ receptor antagonist, bicuculline. Other studies of the same experimental design showed that GABA (13) and (NMDA) (14) also enhance the depressant effects of ethanol upon ICV administration.

In an effort to better understand the nature and mechanism by which glycine enhances the depressant properties of ethanol, the possible modes of action must be considered. The effect of glycine to cause a return to LORR is a drug-induced effect and not related to an osmotic effect of the concentration that was injected. Previous work using the same experimental design showed that isothionic acid (50 μmol/kg) and L-2-oxothiazolidine-4-carboxylic acid (25 μmol/kg) administered ICV showed no significant return to the LORR (14). This is substantiated in the present experiment by the fact that three glycine and three serine solutions of corresponding identical osmolarity were injected ICV and yielded significantly different drug-induced returns to the LORR.

Data from this investigation do not indicate that glycine is causing its effects by inhibiting the biotransformation of ethanol. The inverse relationship between ethanol blood levels and the glycine return to LORR (Table 1) suggest that there is no change in the normal metabolism of ethanol by the animal. Furthermore, the animal blood ethanol concentration fell below the level of ethanol to cause a LORR before the glycine was administered, ruling out the possibility of ethanol causing the return to LORR by itself.

It is important to note that none of the drugs were able to cause a return to LORR when administered in the absence of ethanol at the highest doses used in these experiments with ethanol. Therefore, glycine must have been enhancing the depressant effect of ethanol because it was unable to cause a return to LORR by itself. In addition, a study using identical procedures required μmol/kg doses of GABA to produce a return to LORR (13). It would appear that μmol/kg dosage is required in this ICV injection technique to allow absorption and distribution of the drug from the ventricle of the brain to reach the target tissues in the central nervous system.

The exact mechanism by which glycine enhances the central depressant properties of ethanol cannot be definitively ascertained given the present understanding of the activity of ethanol on the brain. However, glycine has been shown to act on two sites: one is on the NMDA receptor complex and the other is on the chloride ionophore (3,11,28). The NMDA receptor, which is a subgroup of the glutamate receptors, has a binding stie for glycine. This site is a strychnine-insensitive site for glycine binding (28,29). Although a number of in vitro results implicate the involvement of the strychnine-insensitive glycine site as a possible site of the actions of ethanol (21,22,29,38, 39), other studies have shown that the strychnine-insensitive glycine site is fully saturated in vivo (7,22,28). Due to evidence that the glycine modulatory site of the NMDA receptor may be saturated in vivo and the ability of strychnine to antagonize the effect of glycine in a dose-dependent fashion in this present study, the strychnine-insensitive site does not appear to be responsible for this interaction between ethanol and glycine in this present study.

The possible mechanism by which glycine may enhance the

depressant effect of ethanol is by interacting with the strychnine-sensitive glycine site chloride ionophore. The results of the present study show that glycine is able to enhance the ethanol-induced CNS depression, and that this effect is antagonized by strychnine (Table 2). These results indicate that the site of action of glycine is at the strychnine-sensitive receptor, but more information is needed to understand the nature of the interaction. Glycine has been shown to increase chloride conductance in rat cultured spinal cord neurons when administered by itself (19,31). In addition, ethanol has been shown to increase chloride conductance in rat cultured spinal cord neurons when administered by itself (10,34). Therefore, it is possible that ethanol and glycine may act to augment the effect of each other, which would indicate a possible interaction.

The current evidence supporting an interaction between ethanol and glycine is found in a study by Engblom and Akerman (10). Their in vitro experiment on rat synaptoneurosomes showed that ethanol could enhance both GABA and glycine receptor-induced chloride fluxes with physiologically active concentrations. Celentano (6) showed that ethanol augmented glycine- and GABA-induced chloride currents in spinal cord neurons. Previous investigations have shown that strychnine acts selectively as a competitive antagonist at the glycine receptor and has virtually no effect on GABA-induced inhibition (11,16). When all of these factors are considered, it becomes apparent that ethanol may be exerting its depressant effects on the CNS by enhancing glycine chloride fluxes at the strychnine-sensitive chloride ionophore separately and, in addition, to its effects on GABA-mediated chloride fluxes.

In the present study, the GABA antagonist, bicuculline, failed to attenuate the effect of glycine in the presences of ethanol. In previous work (14), the effect of GABA was completely antagonized by bicuculline when similar experiments were performed using the same doses. These results further support the idea that glycine is exerting its ethanol-enhancement effects by acting on the strychnine-sensitive chloride ionophore and not by any GABAergic mechanism.

Serine, an amino acid precursor of glycine, demonstrated an ability to cause a return to the LORR in ethanol-depressed mice. This effect was also antagonized by strychnine. This effect of serine was significantly less than that of glycine and was completely antagonized by strychnine (Table 3). This is consistent with the present understanding that serine has a weak glycine-like action, as demonstrated in iontophoretic studies (27). There is some evidence that serine acts on the strychnine-insensitive site of the NMDA complex (4,30). However, it appears that in this experiment serine exerted its effects weakly at the strychnine-sensitive glycine receptor because it was completely antagonized by strychnine. When serine was injected ICV, the onset of its action to produce a return to the LORR occurred almost immediately. Therefore, the metabolism of serine to glycine by the enzyme, serine hydroxymethyltransferase, would not be responsible for this effect. The $V_{max}$ for this reaction is approximately 15–30 nmol/mg protein/h (2,36). The most likely explanation for the mode of action of serine is a structural similarity to glycine that allowed it to interact with the glycine receptor itself to cause a weak effect on chloride influx.

It would appear that there is an important mechanistic relationship between the amino acid neurotransmitter receptors and ethanol that may help to explain the effects of ethanol. A recent focus of research is on the concept of superfamilies of receptors. The group of amino acids that include GABA, glycine, and glutamate constitutes the most widespread neurotransmitter family in the mammalian central nervous system (8). Data on subunit amino acid sequences and on genetic coding indicate that glycine, GABA, and glutamate receptors all show extensive homology in gene sequence, suggesting that these receptors belong to the same genetic family (37). Furthermore, because activation of NMDA, GABA, and glycine receptors occurs within milliseconds, the ligand binding sites and permeation paths are likely to reside within the same macromolecular complex (8). The findings in the present work that glycine can cause an enhancement of the depressant effect of ethanol at the strychnine-sensitive chloride ionophore were also found to be true for both GABA at its chloride ionophore and NMDA at its receptor in studies of identical experimental design (13,14). It would appear that these findings reinforce the concept of the superfamily of receptors and that ethanol may act on all of these receptor systems to cause its effects in the CNS. Further studies must be done to fully understand ethanol's mechanism of action on the glycine receptor as well as other implicated receptor systems.

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