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BRIEF COMMUNICATION

Interaction Between L-Glutamate and Ethanol on the Central Depressant Properties of Ethanol in Mice

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FERKO, A. P. *Interaction between L-glutamate and ethanol on the central depressant properties of ethanol in mice.* PHARMACOL BIOCHEM BEHAV 47(2) 351–354, 1994.—The effect of L-glutamate to alter ethanol-induced central depression was studies in male Swiss-Webster mice. The duration loss of the righting reflex (LORR) was used as a measurement of CNS depression. Mice were injected (IP) with ethanol (4.0 g/kg), which caused them to lose the righting reflex. After mice regained the righting reflex following ethanol injection (IP), they were immediately injected (ICV) with saline or L-glutamate (1, 15, or 25 μmol/kg). L-Glutamate induced a return to the LORR within 60 s after ICV injection of drug. When L-glutamate was administered (ICV) in the absence of ethanol, no significant loss of the righting occurred. In other experiments, DL-2-amino-5-phosphonovaleric acid (APV), a competitive inhibitor of NMDA, was given ICV with L-glutamate in the presence of ethanol. APV did not significantly antagonize the interaction between ethanol and L-glutamate. When bicuculline methiodide, a GABA antagonists, was administered with L-glutamate (ICV), bicuculline methiodide reduced the effect of L-glutamate to produce a return to the LORR in the presence of ethanol. These data indicate the L-glutamate, an excitatory amino acid neurotransmitter, can enhance the central depressant action of ethanol. It appears that an interaction between the GABAergic and glutamatergic systems may be involved in ethanol intoxication.

Ethanol L-Glutamate Bicuculline CNS depression Loss of righting reflex GABA Sleep time Glutamic acid

THE exact mechanism for the central depressant action of ethanol is unknown, although various neurotransmitters have been investigated. In the past years, some investigators have directed their attention to the involvement of ethanol with amino acid neurotransmitters. Two particular areas that were studied were the inhibitory amino acid GABA and the NMDA receptor associated with the effects of L-glutamate, an excitatory amino acid neurotransmitter.

Evidence suggests that ethanol enhances the activity of the GABAergic system and this interaction between GABA and ethanol may be responsible for the central depressant and anxiolytic properties of ethanol (1,12,17,21). Several reports also have implicated other amino acids in the central action of ethanol. The sulfur-containing amino acid, taurine, which may be a neurotransmitter (14,23), can augment the depressant properties of ethanol (5,10,16). Cysteine, a precursor in the biosynthesis of taurine, and cysteine sulfinic acid, a putative excitatory neurotransmitter (2), are suggested to increase the depressant action of ethanol (6,7).

In addition, NMDA, which activates one of the subgroup of receptors for L-glutamate, can augment ethanol-induced depressant in animals (8). The enhancement of the depressant effect of ethanol by NMDA administration was attenuated by DL-2-amino-5-phosphonovaleric acid (APV), a competitive inhibitor of NMDA, and by administration of bicuculline methiodide, an antagonist of GABA. Therefore, it would seem reasonable to conclude that administration of the neurotransmitter, L-glutamate, should be able to enhance ethanol-induced CNS depression.

In this investigation, L-glutamate is administered ICV in the presence of ethanol. The central depressant action of ethanol is assessed by the measurement of the duration of the loss of the righting reflex (LORR) in mice. The hypothesis of this study is that L-glutamate, an excitatory amino acid, augments the central depressant action of ethanol. Bicuculline methiodide, a GABA antagonist, and APV, an antagonist at the NMDA receptor, are examined in the presence of ethanol and L-glutamate to determine if these antagonists can attenuate

the effect of L-glutamate to enhance the central depressant effect of ethanol.

METHOD

Mice (male Swiss-Webster, 25-30 g) were obtained from Charles River Laboratories (Wilmington, MA). Animals were housed at 21 ± 1°C with a light cycle from 6:00 a.m. to 6:00 p.m. for 1 week prior to the experiments. Mice had free access to Purina Laboratory Chow (Ralston Purina Co., St. Louis, MO) and water. Ethanol solution (20% w/v) for injection (IP) was prepared from 95% ethanol in saline (0.9% NaCl). L-Glutamic acid, APV, and bicuculline methiodide were purchased from Sigma Chemical Co. (St. Louis, MO). Drug solutions for injection (ICV) were prepared in saline and adjusted to pH 7.0 with NaOH solution (5,8,10). All other chemicals were purchased from commercial source and were of analytic grade.

LORR Experiments With Ethanol (IP) and L-Glutamate (ICV)

CNS depression induced by administration of ethanol (IP) was assessed by measuring the duration of the LORR. The duration of the LORR was the time interval between the onset of the LORR after ethanol injection and the gain of the righting reflex. The gain of the righting reflex required that the animal be able to reright itself three times within 15 s after again being placed on its back. In addition, the onset of the LORR (time between ethanol injection and loss of the righting reflex) was recorded. After the animal gained the righting reflex following ethanol injection (IP), it was immediately injected ICV with drug or saline. The second period of the LORR was recorded and called the return to the LORR. The duration of the return to the LORR was measured from the LORR to the gain of the righting reflex after drug or saline injection (ICV).

The procedure (18) for ICV injection into the lateral ventricle involved cutting the scalp of an anesthetized mouse and injecting (at a depth of 3 mm) 2 mm caudal and 2 mm lateral to bregma using a Hamilton microliter syringe (Hamilton Co., Reno, NV) with a 26-ga needle of 3/8 in. Drug solutions were administered slowly into the ventricle over a period of approximately 10 s. The correction position of the injection was verified at autopsy by using trypan blue.

The aim of the following experiments was to determine if L-glutamate could enhance the degree of CNS depression and return animals to a second LORR when it was given ICV at the end of the ethanol-induced LORR.

Animals received an IP injection of ethanol (4.0 g/kg). Twenty minutes after the LORR, a 26-ga needle was used to enter the ventricle of the brain of the ethanol-anesthetized mouse, but no saline or drug solution was given at this time because this was a preparatory step for ICV drug administration (5,6,8). Immediately after animals regained the righting reflex following ethanol administration, they received an ICV injection of saline or L-glutamate (1, 15, or 25 μmol/kg) in a volume of 5 μl. The duration of the return of the LORR was recorded. In this experiment and in all other experiments in this article, blood samples (20 μl) were obtained from the orbital sinus of mice immediately after they regained the righting reflex after ICV injection of saline or drug (5,6,8). An enzymatic method that required alcohol dehydrogenase, nicotinamide adenine dinucleotide (NAD), and spectrophotometry (measuring NADH formation at 340 nm) was employed to determine blood ethanol concentrations (9,15).

Interaction Between L-Glutamate and APV in the Presence of Ethanol

This experiment was done to note if APV was able to attenuate the effect of L-glutamate to enhance the CNS depressant properties of ethanol. Mice were injected with ethanol (4.0 g/kg, IP), and 20 min later a preparatory ICV injection was made. Immediately after animals regained the righting reflex following ethanol administration, they were administered ICV (5 μl) L-glutamate (15 μmol/kg) by itself or a mixture of L-glutamate (15 μmol/kg) and APV (100 nmol/kg). The duration of the return to the LORR was recorded.

In another experiment, L-glutamate was injected ICV in the absence of ethanol to determine if L-glutamate by itself could cause a LORR in mice. Mice were injected with saline (0.02 ml/g, IP) and 20 min later lightly anesthetized with methoxyflurane for the ICV preparatory injection. Fifty minutes after administration of saline, mice were sedated with methoxyflurane (without the LORR) to facilitate the ICV injection (5 μl) with saline or L-glutamate (25 μmol/kg). Mice were observed for 2 h after drug administration.

Interaction Between L-Glutamate Acid and Bicuculline Methiodide

Mice were administered ethanol (4.0 g/kg, IP), and 20 min later a preparatory ICV injection was made as previously described. When animals regained the righting reflex after ethanol injection, they were immediately injected ICV with L-glutamate (15 μmol/kg) by itself or a mixture of L-glutamate (15 μmol/kg) and bicuculline methiodide (10 nmol/kg). The duration of the return to the LORR was recorded.

Statistical Analysis

Significant differences were determined by analysis of variance (ANOVA). All multiple comparisons with a control and comparisons among experimental groups were done by ANOVA followed by Scheffe's test. In the tables, data are expressed as the means ± SE.

RESULTS

Table 1 shows that L-glutamate enhances the central depressant properties of ethanol when various doses of L-glutamate were given by the ICV route in the presence of ethanol. The onset to the return of the LORR following the injection (ICV) of L-glutamate ranged from an immediate effect to a delay of 60 s in which some mice manifested running and jumping behavior before they lost the righting reflex, particularly at the 25-μmol/kg dose.

In the next experiments, L-glutamate was injected in the absence of ethanol to note if the compound by itself could cause a LORR. Mice were administered saline (0.02 ml/kg, IP), and then 50 min later injected ICV with saline or L-glutamate (25 μmol/kg). The saline controls (n = 5) and L-glutamate group (n = 5) lost the right reflex for 0.0 ± 0.0 and 2.2 ± 0.8 min, respectively. Four of the mice in the L-glutamate group exhibited tonic convulsions and/or running and jumping behavior for 15-25 s after the ICV administration of drug. One animal experienced a short seizure (15 s) 5 min after L-glutamate injection. Except for these initial observations, the behavior of the treated group was similar to the control group (saline, ICV) during the 2-h observation period.

In this study, APV, an antagonist at the NMDA receptor, was given with L-glutamate to determine if AVP could attenu-

TABLE 1

EFFECT OF L-GLUTAMATE (GLU) TO PRODUCE A RETURN TO THE LORR IMMEDIATELY AFTER REGAINING THE RIGHTING REFLEX FOLLOWING ETHANOL (ETOH) INJECTION

Group	*n*	Onset to LORR (s)	ETOH-LORR (min)	GLU-Return to LORR (min)*	Blood EtOH (mg/ml)
ETOH† + Saline (controls)	8	88 ± 3	59.4 ± 7.0	1.7 ± 1.2	3.65 ± 0.07
ETOH + GLU (1.0)	7	104 ± 4	47.3 ± 7.5	6.9 ± 1.7	3.78 ± 0.14
ETOH + GLU (15.0)	7	91 ± 3	53.3 ± 9.1	36.7 ± 4.2‡§	3.29 ± 0.14
ETOH + GLU (25.0)	9	92 ± 4	55.9 ± 6.1	25.7 ± 5.4‡#	3.21 ± 0.21

*L-Glutamate injected (μmol/kg, 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 GLU (1.0) group ($p < 0.01$).

#Significantly different from GLU (1.0) group ($p < 0.05$).

ate the effect of L-glutamate at the NMDA receptor, one of the subtypes of glutamate receptors, to which L-glutamate can bind. The data in Table 2 show that APV did not antagonize the effect of L-glutamate. However, bicuculline methiodide, an antagonist of GABA, significantly reduced the duration of the return to the LORR caused by L-glutamate in the presence of ethanol (Table 2). Previous works using in vitro experiment have demonstrated that activation of the NMDA, kainate, and quisqualate receptors can cause the release of GABA from neuronal tissues. (4,11). When only bicuculline (10 nmol/kg, ICV) was administered ICV in the presence of ethanol, no significant second LORR (return to the LORR) was observed (data not shown). In addition, when APV (100 nmol/kg, ICV) was injected by itself into mice in the presence of ethanol the compound caused a slight increase in the return to the LORR but this was not significant (data not shown). Similar results for APV have been published previously (8).

DISCUSSION

This study shows that L-glutamate, an excitatory amino acid, enhances the central depressant properties of ethanol. This effect of L-glutamate in the presence of ethanol is similar to the effect of NMDA (8). In addition, the amino acid cysteine sulfinic acid also augments ethanol-induced depression as measured by the LORR. Cysteine sulfinic acid can cause the release of GABA, and the interaction between ethanol and this amino acid was attenuated by bicuculline methiodide, a GABA antagonist (7).

In this present investigation, μmol/kg dosage of L-glutamate was required for ICV injection. This dosage range seems to be necessary for the absorption and distribution of the drug to its site of action once L-glutamate was deposited into the ventricle of the brain. In another work, a similar dosage in the μmol/kg range was needed for GABA, an inhibitory neurotransmitter, to enhance the depressant effect of ethanol when GABA was administered by the ICV route in the same types of experiments (6,7).

In this study, the interaction between L-glutamate and ethanol appears to involve the GABAergic system in the CNS. Receptors for glutamate are present on GABAergic neurons (4). The agonists, NMDA, kainate, and quisqualate, for the subgroups of the glutamate receptors can cause the release of GABA in cultured neuronal cells and quisqualate receptor activation produces the most potent effect to release GABA (4,11). It is suggested that the effect of glutamate to release GABA may occur primarily through the non-NMDA receptors although NMDA can promote GABA release (11). In that study, an NMDA antagonist completely blocked NMDA-induced release of GABA but the antagonist only partially

TABLE 2

EFFECT OF BICUCULLINE METHIODIDE (BIC) AND APV ON THE DURATION OF THE RETURN OF THE LORR BY L-GLUTAMATE (GLU) IN THE PRESENCE OF ETHANOL (ETOH)

Group*	*n*	Onset to LORR (s)	ETOH-LORR (min)	GLU-Return to LORR (min)	Blood ETOH (mg/ml)
BIC + GLU					
ETOH† + GLU	10	92 ± 3	55.8 ± 4.5	26.1 ± 4.6	3.21 ± 0.12
ETOH + GLU + BIC	10	95 ± 3	51.4 ± 4.2	8.9 ± 2.6‡	3.51 ± 0.13
APV and GLU					
ETOH + GLU	10	96 ± 3	55.0 ± 3.7	22.5 ± 2.2	3.53 ± 0.09
ETOH + GLU + AVP	6	92 ± 4	54.5 ± 7.3	26.8 ± 4.2	3.32 ± 0.11

*GLU (15 μmol/kg, ICV), BIC (10 nmol/kg, ICV), AVP (100 nmol/kg, ICV). GLU alone or with BIC or AVP was injected (ICV) immediately after regaining the righting reflex following ETOH administration.

†ETOH was given 4.0 g/kg IP.

‡Significantly different from corresponding ETOH + GLU group ($p < 0.05$).

reduced the effect of glutamate to release GABA from neurons in primary cell culture (11). In another study, APV administration (ICV) significantly attenuated the effect of NMDA to enhance the depressant action of ethanol (8). In this study, Table 2 shows that APV at a similar dose did not reduce the effects of L-glutamate to augment ethanol-induced CNS depression. These results indicate that other receptors for glutamate besides the NMDA receptor are involved in the release of GABA (4,11). Previous work also showed that bicuculline methiodide, a GABA antagonist, decreases the interaction between NMDA and ethanol (8). The results from the experiments in this present investigation suggest that L-glutamate stimulates its subgroups of receptors (22) to promote the release of GABA in the CNS and thereby enhances the depressant action of ethanol because bicuculline methiodide antagonizes the effect of L-glutamate in the presence of ethanol (Table 2).

In addition, another factor may contribute to this interaction between ethanol and L-glutamate. L-Glutamate can be converted into GABA in the CNS (13). The biosynthesis of GABA from L-glutamate is an enzyme-mediated reaction. It seems, however, that the formation of GABA from L-glutamate would at most play a minor role in the enhancement of the depressant action of ethanol because sufficient time would be needed for the synthesis of GABA and this reaction is not a spontaneous reaction. The onset of the action of L-glutamate after ICV injection in the presence of ethanol to induce a return to the LORR was short. It occurred within 60 s or less. In a number of animals, the onset of the effect of L-glutamate was immediate.

Several reports provide evidence for an interaction between the inhibitory neurotransmitters, GABA, and the excitatory neurotransmitter glutamate in the CNS (3,19). Others have shown that L-glutamate, kainate, and quisqualate can enhance GABA-dependent chloride uptake in the cortex (20). The intoxication induced by ethanol is indicated to involve a relationship between ethanol and the GABAergic system (1, 12,17,21). Previous work also suggested that NMDA enhanced the central depressant properties of ethanol by an interaction between the GABAergic and glutamatergic systems (8). The results of this present investigation also support an interaction between the GABAergic and glutamatergic systems for the acute effect of ethanol. Although GABA and glutamate are implicated in the depressant action of ethanol, it appears that further experimentation is necessary to more fully understood the complex mechanism of action that is involved with ethanol and the neurotransmitters, GABA and glutamate, when experiments are done in vivo.

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