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BRIEF COMMUNICATION

NMDA Enhances the Central Depressant Properties of Ethanol in Mice

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FERKO, A. P. *NMDA enhances the central depressant properties of ethanol in mice.* PHARMACOL BIOCHEM BEHAV 43(1) 297–301, 1992.—Male Swiss-Webster mice were used to examine the effect of NMDA on the ethanol-induced loss of the righting reflex (LORR). The LORR was used as a measure of CNS depression. Immediately after animals regained the righting reflex following ethanol injection (4.0 g/kg, IP) mice received an ICV injection of saline or NMDA (10, 50, 100, or 500 nmol/kg) in a volume of 5 μl. Upon ICV injection of NMDA, mice again lost the righting reflex and this effect of NMDA in the presence of ethanol occurred rapidly and in a dose-dependent manner. In another experiment DL-2-amino-5-phosphonovaleric acid (APV), a competitive antagonist of NMDA, was given ICV with NMDA (50 nmol/kg) in the presence of ethanol. APV (10 and 100 nmol/kg, ICV) significantly attenuated the response of NMDA to enhance the depressant action of ethanol. When bicuculline methiodide, an antagonist of GABA, was given ICV with NMDA (50 nmol/kg), bicuculline methiodide reduced the effect of NMDA to produce a second loss of the righting reflex (return to the LORR) in the presence of ethanol. When NMDA (100 nmol/kg, ICV) was injected in the absence of ethanol into mice, NMDA by itself did not produce a loss of the righting reflex. In this investigation, the results suggest that NMDA can augment ethanol-induced depression possibly through an interaction between glutamatergic and GABAergeric systems in the CNS.

Ethanol NMDA Bicuculline CNS depression Loss of righting reflex GABA Sleep time

SEVERAL investigations have shown that NMDA and glutamate can cause the release of GABA from cultured neurons (6,15,29). It appears that receptors for endogenous glutamate are present directly on the GABAergic neurons (6). In addition, another excitatory amino acid, cysteine sulfinic acid (5), which is believed to be a putative excitatory amino acid, can also release GABA from brain slices through a possible receptor mechanism (1,2,25). Recent in vivo work shows that cysteine sulfinic acid enhances the central depressant effect of ethanol in mice and that this effect of cysteine sulfinic acid is reduced by bicuculline, a GABA antagonist (9). Because NMDA can release GABA during in vitro experiments, it may be that this compound can alter the depressant action of ethanol. Previous works have reported that GABA and GABA agonists increase the depressant action of ethanol as measured by the loss of the righting reflex (LORR) (8,19,23).

In this investigation, NMDA is administered ICV in the presence of ethanol. The interaction between NMDA with DL-2-amino-5-phosphonovaleric acid (APV), an antagonist of NMDA, or bicuculline methiodide, an antagonist of GABA, is examined in the presence of ethanol. The hypothesis of this investigation is that NMDA augments the depressant action of ethanol. The LORR is used to measure the degree of CNS depression produced by ethanol.

METHOD

Male Swiss-Webster mice (25–30 g) were obtained from Charles River Laboratories (Wilmington, MA). Mice were housed at 21 ± 1°C with a light cycle from 6:00 a.m.–6:00 p.m. for 1 week prior to experimentation. Animals had free access to Purina Laboratory Chow (Ralston Purina Co., St. Louis, MO) and water. Ethanol solution (20% w/v) for injection was prepared from 95% ethanol in saline. NMDA, APV, and bicuculline methiodide were purchased from Sigma Chemical Co. (St. Louis, MO). Drug solutions for injection (ICV) were prepared in saline (0.9% NaCl) and adjusted to pH 7.0 with NaOH solution (7,8,11). All other chemicals were purchased from commercial sources and were of analytical grade.

LORR Experiments with Ethanol (IP) and NMDA (ICV)

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

The duration of the LORR was used as an index of ethanol-induced CNS depression, and was measured as the interval between the LORR after ethanol injection (IP) 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. This 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 (27) for ICV injection 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 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 correct position of the injection was verified at autopsy by using trypan blue.

In these experiments, mice received an IP injection of ethanol (4.0 g/kg). Twenty minutes after the LORR, 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 the time because this was a preparatory step for ICV drug administration (7,8,11). Immediately after animals regained the righting reflex following ethanol administration, mice received an ICV injection of saline or NMDA (10, 50, 100, or 500 nmol/kg) in a volume of 5 μl. The duration of the return to the LORR was recorded. In this experiment and in all other experiments in this study, blood samples (20 μl) were obtained from the orbital sinus of mice when they regained the righting reflex after ICV injection of saline or drug. An enzymatic method that required alcohol dehydrogenase, nicotinamide adenine dinucleotide (NAD), and spectrophotometry (measured NADH formation at 340 nm) was used to assess blood ethanol concentrations (10,22).

Interaction Between NMDA and APV in the Presence of Ethanol

It was decided to examine first the effects of APV alone in the presence of ethanol to determine if APV could produce a LORR when given by the ICV route. Mice were given an IP injection of ethanol and 20 min after administration of ethanol a preparatory ICV injection was made as previously described. When mice regained the righting reflex after ethanol administration, they were immediately injected ICV (5 μl) with APV (1, 10, 100, or 1000 nmol/kg). The duration of the return of the LORR was recorded.

In the next experiment, animals were administered ethanol (4.0 g/kg, IP), and 20 min later a preparatory ICV injection was made. Immediately after mice regained the righting reflex following ethanol administration, they were injected ICV (5 μl) with NMDA (50 nmol/kg) by itself or a mixture of NMDA (50 nmol/kg) and APV (10 or 100 nmol/kg). The duration of the return to the LORR was recorded.

In another experiment, NMDA or APV was injected ICV in the absence of ethanol to note if NMDA or APV 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. At this time, an ICV preparatory injection was made. Fifty minutes after administration of saline, mice were heavily sedated with methoxyflurane (without the LORR) and injected (5 μl) with saline, NMDA (100 nmol/kg), or APV (1000 nmol/kg). Mice were observed for 2 h after drug administration.

Antagonism of NMDA by Bicuculline Methiodide

Mice were injected with 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 administration, they were immediately injected ICV (5 μl) with NMDA (50 nmol/kg) by itself or with bicuculline methiodide (10 or 50 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 comparison among experimental groups were done by ANOVA followed by Scheffe's test. In the tables, data are expressed as the means ± SE.

RESULTS

Under the experimental design of this study, NMDA, which stimulates one of the subgroups of the glutamate receptors, caused an augmentation of the central depressant properties of ethanol in a dose-dependent manner (Table 1). The onset of the return to the LORR varied from immediate to 60 s when NMDA was injected ICV in the presence of ethanol. Some animals exhibited an initial excitatory behavior manifested by excessive running and jumping at 50 nmol/kg, as well as momentary convulsions at 100 nmol/kg. When NMDA was injected ICV at 500 nmol/kg, 50% of the group died (data not shown).

In this study, APV was injected (ICV) into mice following the regaining of the righting reflex after ethanol administration. This experiment was done to determine if APV by itself could cause a return to the LORR in the presence of ethanol. At the end of the LORR after ethanol administration, mice (n = 6–8) were injected (ICV) with saline (controls) or APV. The saline group and APV groups at 1, 10, 100, and 1000 nmol/kg lost the righting reflex for 1.9 ± 1.0, 6.6 ± 20, 18.3 ± 7.0, 12.3 ± 2.4, and 47.8 ± 4.7 min, respectively. APV did produce a slight increase in the duration of the return to the LORR; however, only the highest dose (1000 nmol/kg) of APV caused a significant ($p < 0.01$) return to the LORR in the presence of ethanol. Therefore, the 10- and 100-nmol/kg doses of APV were selected for the interaction experiments with NMDA. Table 2 shows that APV (10 and 100 nmol/kg) significantly attenuated the response of NMDA and, therefore, it appears that NMDA is binding to the NMDA receptor to enhance the depressant effect of ethanol.

In previous in vitro works, others have shown that NMDA can cause the release of GABA from neuronal tissues (6,15, 29). Table 2 also indicates that bicuculline methiodide (50 nmol/kg), a GABA antagonist, antagonized the duration of the return to the LORR produced by NMDA in the presence of ethanol. When only bicuculline (10 and 50 nmol/kg, ICV) was injected in the presence of ethanol, no significant second LORR (return to the LORR) was observed (data not shown).

In the next experiments, NMDA or APV was administered (ICV) into mice in the absence of ethanol to determine if either agent by itself could cause a LORR. Mice were administered saline (0.02 ml/g, IP) and then 50 min later injected (ICV) with saline, NMDA (100 nmol/kg), or APV (1000 nmol/kg). The controls (n = 3, saline), NMDA group (n = 4), and APV group (n = 4) lost the righting reflex for 0.3 ± 0.2, 0.9 ± 0.5, and 0.0 ± 0.0 min, respectively. The NMDA group experi-

TABLE 1

INTERACTION BETWEEEN ETHANOL (ETOH) AND NMDA ON THE DURATION OF THE RETURN TO THE LORR

Group	n	Onset to LORR (seconds)	ETOH LORR (min)	NMDA Return* to LORR (min)	Blood ETOH (mg/ml)
ETOH† + saline (controls)	8	90 ± 3	55.1 ± 4.3	1.6 ± 1.2	3.60 ± 0.07
ETOH + NMDA (10)	6	95 ± 5	56.1 ± 5.8	10.2 ± 3.9	3.50 ± 0.07
ETOH + NMDA (50)	7	99 ± 4	48.4 ± 3.4	22.0 ± 2.9‡	3.24 ± 0.12
ETOH + NMDA (100)	6	98 ± 6	58.3 ± 7.7	33.0 ± 7.1‡§	3.12 ± 0.08‡

*NMDA injected (nmol/kg, ICV) immediately after regaining the righting reflex following ETOH administration.

†ETOH was given 4.0 g/kg IP.

‡Significantly different from control group ($p < 0.01$).

§Significantly different from ETOH + NMDA (10) group ($p < 0.01$).

enced vigorous jumping and running for 10 s in 50% of animals. The APV group did not lose the righting reflex; however, they did exhibit a degree of sedation and motor incoordination that lasted between 12 and 22 min. In all other behavioral effects, the treated groups were quite similar to the controls during the 2-h observation period following ICV administration of drugs.

DISCUSSION

The results of this investigation show that NMDA, an excitatory agonist on a subtype of glutamate receptors, enhances the depressant properties of ethanol. There is an inverse relationship between blood ethanol concentrations and the duration of the NMDA return to LORR (Table 1), indicating that biotransformation of ethanol is not altered (9). Similar results to the interaction between NMDA and ethanol were also previously obtained when cysteine sulfinic acid, an excitatory amino acid, was administered (ICV) in the presence of ethanol in the same types of experiments (9).

The exact mechanism of action for the effect of NMDA to return animals to a second LORR following ethanol injection is not known. It seems, however, that both NMDA and GABA receptors are involved because both APV, an antagonist of NMDA, and bicuculline methiodide, an antagonist of GABA, attenuated the effect of NMDA in the presence of ethanol (Table 2). GABAergic neurons possess receptors on them for excitatory amino acids such as the glutamate subtype receptor for NMDA (5,16). Activation of NMDA receptors on GABAergic neurons can lead to transmembrane movement of sodium, potassium, and calcium ions and subsequent release of GABA (6,15,29). Other investigators have indicated that some of the effects of ethanol in the CNS are related to the GABAergic system (23,30). In addition, reports have shown that acute ethanol administration in vitro causes the release of L-glutamate from rat striatal, midbrain, and cerebral cortical slices (24,26).

Recent works suggest that acutely administered ethanol may actually inhibit activity of the NMDA receptor in vivo

TABLE 2

APV AND BICUCULLINE METHIODIDE (BIC) ANTAGONIZE THE DURATION OF THE RETURN TO THE LORR INDUCED BY NMDA IN THE PRESENCE OF ETHANOL (ETOH)

Group	n	Onset to LORR (seconds)	ETOH LORR (min)	NMDA Return* to LORR (min)*†	Blood ETOH (mg/ml)
AVP and NMDA					
ETOH‡ + NMDA	11	95 ± 3	50.8 ± 2.4	21.4 ± 2.1	3.46 ± 0.09
ETOH + NMDA + APV (10)	7	98 ± 5	41.8 ± 5.6	10.7 ± 4.0§	3.26 ± 0.14
ETOH + NMDA + APV (100)	6	95 ± 3	44.7 ± 2.8	8.8 ± 2.3§	3.39 ± 0.10
BIC and NMDA					
ETOH + NMDA	9	98 ± 3	50.3 ± 3.0	22.3 ± 2.5	3.46 ± 0.09
ETOH + NMDA + BIC (10)	6	93 ± 4	42.8 ± 3.4	20.5 ± 4.2	3.27 ± 0.09
ETOH + NMDA + BIC (50)	7	96 ± 4	49.8 ± 4.2	4.4 ± 1.6§	3.62 ± 0.06

*NMDA (50 nmol/kg, ICV), APV (10 and 100 nmol/kg, ICV), or NMDA plus APV was injected (ICV) immediately after regaining the righting reflex following ETOH administration.

†NMDA (50 nmol/kg, ICV), BIC (10 and 50 nmol/kg, ICV), or NMDA plus BIC 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 + NMDA group ($p < 0.05$).

because chronic administration of ethanol to animals shows an increase in the number of NMDA receptors, reflecting an upregulation of receptors due to the chronic inhibitory action of ethanol (14,17). In these experiments, ligand binding to the NMDA receptors increased in the hippocampal tissue but not in cerebral cortex. In addition, autoradiography of MK-801 binding to NMDA receptors showed that binding was highest in hippocampal and some increase was seen in cortical areas, striatum, and thalamus, but not in hypothalamus and cerebellum. Another study (3) shows somewhat different results in that chronic ethanol administration to animals actually lowers glutamate binding to NMDA receptors rather than increases binding to NMDA receptors. These investigators reported a decrease in glutamate binding to NMDA receptors in rat hippocampal tissue and also a decrease in glutamate binding to NMDA receptors in human postmortem hippocampal sections from chronic alcohol abusers.

Reports have indicated that ethanol inhibits NMDA-stimulated release of norepinephrine (12,13) and dopamine (31). Calcium ion fluxes and cyclic guanosine monophosphate production mediated by NMDA are attenuated by ethanol (18). In voltage-clamped hippocampal neurons, ethanol reduces ion currents induced by NMDA (21). Ethanol inhibits NMDA-stimulated increase in intracellular calcium concentration (4). In studies with NMDA-activated single-channel currents in cell culture, low doses of ethanol potentiate the NMDA effect while higher concentrations of ethanol block the NMDA-activated single-channel currents (20). It is suggested that the effect of ethanol on NMDA receptors may produce an imbalance in neurotransmission that may have some contribution to the intoxicating effects of ethanol in vivo (13,21).

Various studies are present in the literature on the in vitro effects of ethanol and the NMDA receptor; however, few experiments examine the effects of ethanol and NMDA in an animal model. It is known that in vitro experiments sometimes may not reflect the in vivo situation (28). To understand the involvement of NMDA receptors in the effects of ethanol, in vivo studies of ethanol on NMDA-mediated processes are necessary (13). Although acute administration of ethanol in in vitro experiments can inhibit or stimulate some effects of NMDA, it appears that in this study with the whole animal the augmentation of the depressant properties of ethanol by NMDA is the end result of the total summation of all the effects of NMDA (both excitatory and inhibitory) in the CNS. Because the relationship between the glutamatergic and GABAergic systems in the presence of ethanol may be more complex, further experimentation must be done to delineate more fully the mechanism of action of ethanol and its involvement with the NMDA receptor.

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