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Effects of Milacemide, a Glycine Prodrug, on Ethanol's Antiseizure Efficacy

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DEUTSCH, S. I., D. O. NORRIS, D. A. O'CONNOR, M. R. NOVITZKI, L. G. LUKACS AND J. MASTROPAOLO. *Effects of milacemide, a glycine prodrug, on ethanol's antiseizure efficacy.* PHARMACOL BIOCHEM BEHAV **41**(2) 263-266, 1992. — A variety of in vitro data suggest that ethanol interferes with *N*-methyl-D-aspartate (NMDA)-stimulated calcium ion conductance. This effect occurs at ethanol concentrations in blood associated with acute intoxication in the nontolerant human (< 50 mM) and may involve its selective action at the strychnine-insensitive glycine binding site on the NMDA receptor complex. Moreover, there are in vitro data showing that glycinergic interventions can attenuate ethanol's inhibitory actions on NMDA-mediated transmission. The relevance of these in vitro findings to the intact animal was tested in an incremental electroconvulsive shock (IECS) paradigm using milacemide, a lipophilic prodrug of glycine. In this paradigm, the influence of milacemide on ethanol's ability to antagonize the electrical precipitation of seizures was tested. Doses of 3.2 and 32.0 mg/kg did not change ethanol's antiseizure efficacy, whereas 320.0 mg/kg potentiated ethanol's antiseizure efficacy. The mechanism of potentiation of ethanol's antiseizure efficacy by milacemide is unknown. Potentiation could result from stimulation of chloride ion conductance in the brainstem by glycine liberated from the lipophilic prodrug and acting at the strychnine-sensitive site. Alternatively, unmetabolized milacemide, which accumulates at the highest administered dose, may antagonize NMDA-mediated neural transmission. The latter explanation would be consistent with a role for receptor-gated calcium ion conductance in the mediation of ethanol's actions.

Milacemide Ethanol Seizure Glycine NMDA receptor

ETHANOL has been shown to interfere with calcium ion conductance through receptor-gated channels at concentrations in blood associated with acute intoxication in the nontolerant human (2,3). For example, ethanol attenuated the ability of *N*-methyl-D-aspartic acid (NMDA), an L-glutamic acid analogue, to activate cationic conductance in voltage-clamped hippocampal neurons (5). The IC_{50} for this inhibitory effect of ethanol on the amplitude of the inward current was about 30 mM, and a threshold effect was observed at about 5 mM. Ethanol also attenuated calcium ion influx into cultured cerebellar granule cells (5). The IC_{50} for this effect of ethanol on calcium ion flux was 41 mM. Glycine and D-serine, a glycine agonist acting at the strychnine-insensitive site, were able to overcome this antagonistic action of ethanol (10). Ethanol was also shown to inhibit NMDA-stimulated striatal release of dopamine in a slice preparation; the IC_{50} for this effect of ethanol was about 21 mM (14). Exogenous glycine was able to overcome ethanol's inhibition of striatal dopamine release in a dose-dependent manner. The data suggest that ethanol may interfere with the glycine modulatory site and that exogenous glycine may attenuate some of ethanol's actions.

Ethanol's interference with NMDA-stimulated calcium ion conductance in vitro appears to be relevant to some of its acute effects in the intact animal (3,12). For example, MK-801 ([(+)-5-methyl]-10,11-dibenzo [*a,d*] cyclohepten-5,10-imine maleate), a noncompetitive allosteric antagonist of NMDA-mediated neural transmission (13), potentiated ethanol's antiseizure efficacy at a dose that was itself devoid of antiseizure activity (3). Moreover, indole-2-carboxylic acid (I2CA; 100 mg/kg, IP) and D-cycloserine (320 mg/kg, IP), ligands acting at the strychnine-insensitive glycine binding site, also potentiated ethanol's antiseizure efficacy (3). I2CA is a known competitive antagonist of glycine, and D-cycloserine is a known partial agonist at the strychnine-insensitive site (6,7). Finally, phencyclidine (PCP) and MK-801 substitute for ethanol as a discriminative stimulus in pigeons trained to discriminate between ethanol and water (12). PCP is a noncompetitive allosteric antagonist of NMDA-stimulated calcium ion conductance that binds to the same channel site as MK-801. Thus, interference with NMDA-stimulated calcium ion conductance may be relevant to ethanol's behavioral actions in the intact animal.

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The NMDA receptor complex is a receptor-gated cation channel that, in addition to the agonist recognition site for L-glutamic acid, contains distinct recognition sites for endogenous and exogenous modulatory ligands (13). Glycine binding to a strychnine-insensitive site on the NMDA receptor complex potentiates the ability of L-glutamic acid to promote calcium ion conductance. We hypothesize that facilitation of NMDA-mediated neural transmission by stimulation of the strychnine-insensitive glycine binding site may attenuate some of ethanol's action in the intact animal. Recently, it has been shown that intracerebroventricular administration of the glycine agonist, D-serine, potentiated the ability of racemic *N*-methyl-aspartic acid to precipitate seizures in mice (11). These data show that NMDA receptors may not be maximally potentiated by glycine in vivo. Further, these data support the development of drugs acting at the strychnine-insensitive glycine binding site to potentiate NMDA-mediated neural transmission. In this study, we examined the ability of milacemide (2-*n*-pentylaminoacetamide), a lipophilic prodrug of glycine, to attenuate ethanol's antiseizure efficacy in an incremental electroconvulsive shock procedure. Milacemide readily crosses the blood–brain barrier and is then converted to glycinamide and glycine subsequent to its deacylation by monoamine oxidase B (1,8).

METHOD

Animals

Experimentally naive, male NIH Swiss mice weighing approximately 25 g were used throughout the experiments.

Drugs

Milacemide was obtained from G.D. Searle & Co. Absolute ethanol (USP 200 proof) was obtained from the Florida Distillers Company (Alfred, FL). Milacemide and absolute ethanol were dissolved in distilled, deionized water. All drugs and vehicles were prepared on each day of the experiment and injected intraperitoneally in a volume of 0.01 ml/g body weight.

Incremental Electroconvulsive Shock Procedure

In the incremental electroconvulsive shock (IECS) procedure, a Hittman electroconvulsive shock generator (Medcraft model B24-III) was utilized to administer 0.3 s of voltage via earclip electrodes. The procedure began with 70 V and was increased in 10-V increments every 2 s until a full seizure (maximal tonic hindlimb extension) occurred or 170 V was reached. This procedure was approved by the Animal Studies Subcommittee and Research Committee of this Department of Veterans Affairs Medical Center. The antiseizure efficacy of ethanol was assessed in groups receiving injections of either vehicle (distilled water) or ethanol (0.56, 0.75, 1.0, 1.34, and 1.8 g/kg) 20 min prior to the IECS procedure. Milacemide (3.2, 32.0, and 320.0 mg/kg) or its vehicle (distilled water) was injected 40 min prior to ethanol and its vehicle. Groups of at least 10 mice were tested in each of the experimental conditions.

Determination of Blood Ethanol Concentrations

Blood ethanol concentrations were determined using a commercially available spectrophotometric procedure [Sigma Diagnostics Procedure No. 332-UV; see (9)]. Briefly, according to the original method, aliquots of plasma containing unknown concentrations of ethanol were added to a reaction mixture containing yeast alcohol dehydrogenase (ADH, 150 units) and nicotinamide adenine dinucleotide (NAD, 1.8 μmol). The reaction proceeded for 10 min at room temperature. The amount of NAD reduced was measured at 340 nm. Blood ethanol levels were calculated from a standard curve (0–300 mg/dl) that was analyzed simultaneously with the samples.

Data Analysis

Each of the three experiments was subjected to a between-subjects two-way analysis of variance using ethanol dose and milacemide dose as between-subjects factors. An overall analysis of the data was not done because of the variability inherent in the ethanol dose-response curve replications. For the purpose of data analysis, animals that did not seize were assigned a voltage of 180.

RESULTS

As shown in Fig. 1, a significant main effect was observed for ethanol ($p < 0.05$). In a dose-dependent manner, ethanol raised the threshold voltage for seizure production in the IECS paradigm. This dose-response relation between ethanol and threshold voltage for seizure production was not altered in

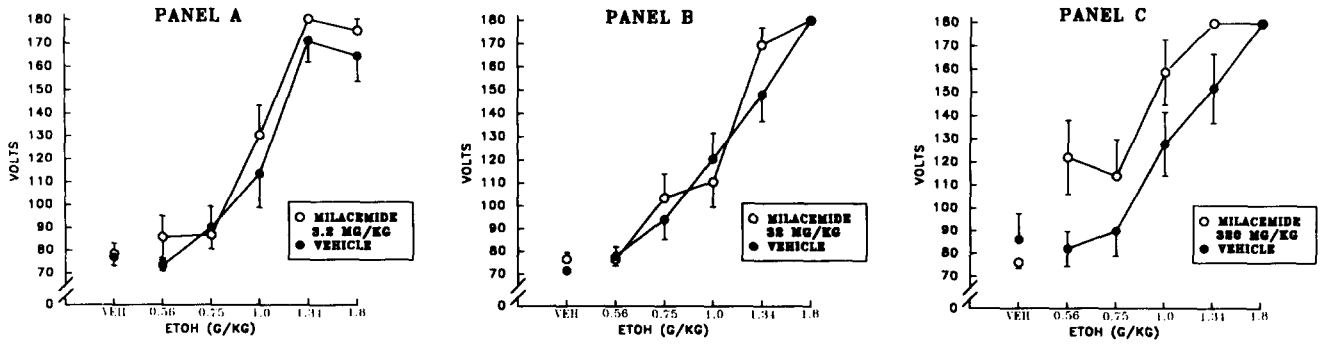

FIG. 1. Mean voltages for producing seizures in groups of mice injected with either milacemide (panel A, 3.2 mg/kg; panel B, 32.0 mg/kg; panel C, 320.0 mg/kg; open circles) or vehicle (closed circles) 40 min prior to injection of one of five doses of ETOH (0.56, 0.75, 1.0, 1.34, 1.8 g/kg) or its vehicle, injected 20 min prior to IECS testing.

TABLE 1

MEAN BLOOD ALCOHOL CONCENTRATIONS (mg%) FOR FOUR GROUPS OF MICE RECEIVING ETHANOL (1.34 g/kg) AND EITHER ONE OF THREE DOSES OF MILACEMIDE (3.2, 32.0, OR 320.0 mg/kg) OR ITS VEHICLE

		Milacemide (mg/kg)		
	VEH	3.2	32.0	320.0
Mean (mg%)	111.45	131.09	128.89	131.16
SD	31.658	12.287	12.452	18.244

groups of mice receiving either 3.2 or 32.0 mg/kg milacemide compared with groups receiving vehicle. However, milacemide potentiated ethanol's antiseizure efficacy in groups pretreated with 320 mg/kg, $F(1,108) = 8.837, p = 0.0036$.

To determine whether 320.0 mg/kg milacemide raised the ethanol concentration in blood, thereby potentiating ethanol's antiseizure efficacy, alcohol concentrations were examined in plasma derived from uncoagulated, whole trunk blood. Four groups of mice ($n = 10$) treated with 1.34 g/kg ethanol (ethanol alone, and ethanol and milacemide at doses of 3.2, 32.0, and 320.0 mg/kg) were tested. Trunk blood samples were collected from each animal 20 min after ethanol injection; as per the above experiments, milacemide was injected 40 min prior to ethanol. Blood alcohol concentrations did not differ between the four groups (see Table 1). Thus, milacemide's ability to potentiate ethanol's antiseizure efficacy was not due to its interference with the metabolism or clearance of ethanol.

DISCUSSION

Potentiation of ethanol's effects by interference with receptor-gated calcium ion conductance in the intact animal has been documented (3,12). For example, I2CA, D-cycloserine, and MK-801 have been shown to potentiate ethanol's antiseizure efficacy in the IECS paradigm (3). Furthermore, noncompetitive NMDA antagonists (i.e., PCP, MK-801, and ketamine) can substitute for ethanol as interoceptive cues in a drug discrimination paradigm (12).

The exogenous administration of glycine and D-serine, a glycine analog, attenuated ethanol's inhibitory effects on calcium ion conductance and NMDA-stimulated striatal dopamine release in vitro (10,14). Also, D-serine was able to potentiate the ability of racemic *N*-methyl-aspartic acid to precipitate seizures in intact mice (11). Thus, the NMDA receptor complex in intact animals may not be maximally stimulated by levels of endogenous glycine. In this study, "low doses" of milacemide did not alter ethanol's antiseizure efficacy, whereas a "high dose" potentiated this effect. There are data to suggest that high-dose milacemide may actually interfere with NMDA-mediated neural transmission (4). According to this view, high-dose administration results in the accumulation of unmetabolized levels of milacemide, which possesses antagonistic properties at the NMDA receptor complex.

In summary, milacemide did not attenuate ethanol's antiseizure efficacy in the IECS paradigm. Moreover, the highest dose of milacemide examined in this study (320.0 mg/kg) potentiated ethanol's efficacy. High-dose milacemide administration is associated with accumulation of the unmetabolized parent compound, which is thought to possess antagonistic effects at the NMDA receptor complex (4). Potentiation of ethanol's effects by antagonism of NMDA-mediated neural transmission may be consistent with a role for receptor-gated calcium ion conductance in at least some of ethanol's actions. The potentiation of ethanol's antiseizure efficacy by milacemide could also be accounted for by the ability of liberated glycine to stimulate chloride ion conductance at strychnine-sensitive sites in brain stem. Future work should explore the ability of milacemide to attenuate ethanol's effects in other behavioral paradigms. The possibility that a centrally effective "glycine agonist" that crosses the blood–brain barrier can attenuate some of ethanol's acute effects is an attractive one.

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