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Potentiation of Ethanol Via Interference With Calcium Channels

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DEUTSCH, S. I., M. KAUSHIK, J. A. HUNTZINGER, M. R. NOVITZKI AND J. MASTROPAOLO. *Potentiation of ethanol via interference with calcium channels.* PHARMACOL BIOCHEM BEHAV **38**(3) 665–668, 1991.—The relevance of ethanol's ability to inhibit voltage-gated and receptor-gated calcium ion conductance in vitro to its acute effects in intact animals was examined. Specifically, nimodipine (a voltage-sensitive calcium antagonist of the dihydropyridine class), indole-2-carboxylic acid (a competitive antagonist of the strychnine-insensitive glycine binding site), MK-801 (a noncompetitive allosteric antagonist of the NMDA receptor complex), and d-cycloserine (a partial agonist of the strychnine-insensitive glycine binding site) were examined for their ability to alter ethanol's antiseizure efficacy in an incremental electroconvulsive shock paradigm. The results showed that drugs known to interfere with voltage-gated and receptor-gated calcium ion conductance potentiated ethanol's antiseizure efficacy. These results implicate voltage-gated and receptor-gated calcium ion conductance in ethanol's acute pharmacologic effects in intact animals.

Ethanol Antiseizure efficacy Voltage-sensitive calcium channels NMDA receptor Nimodipine
Indole-2-carboxylic acid MK-801 d-Cycloserine

ETHANOL has been shown to interfere with calcium ion conductance through voltage-gated and neurotransmitter receptor-gated mechanisms (1). For example, the "fast-phase" of depolarization-dependent calcium ion uptake into synaptosomes and cultured neurons, which occurs through voltage-sensitive L-type calcium channels, is inhibited by ethanol (5, 11, 17). Moreover, chronic exposure of animals and cultured neurons to ethanol results in an upregulation of the binding site for the dihydropyridine class of voltage-sensitive calcium-channel antagonist (3, 4, 12, 13, 15, 16). This latter effect of chronic ethanol exposure could be responsible for some of the withdrawal-emergent adverse effects in the ethanol-tolerant/dependent individual, especially seizures. Ethanol has also been shown to interfere with the ability of *N*-methyl-D-aspartate (NMDA), an excitatory amino acid analogue, to activate currents in voltage-clamped hippocampal neurons and stimulate calcium ion influx into cultured cerebellar granule cells (6,14). The above effects of ethanol occur at concentrations that are associated with acute intoxication in the nontolerant human. Thus the suggestion has been made that ethanol's ability to interfere with the elevation of intraneuronal calcium ion concentrations via voltage-gated and receptor-gated mechanisms is involved in the mediation of its acute intoxicational properties (1).

The current investigation was undertaken to examine the functional relevance of ethanol's effects on voltage-gated and receptor-gated calcium ion conductance in the intact animal. Specifically, an incremental electroconvulsive shock (IECS) procedure was employed to assess the effect of nimodipine, a voltage-sensitive calcium antagonist of the dihydropyridine class, and drugs which interact selectively with the NMDA receptor complex on ethanol's antiseizure efficacy (2). In addition to nimodipine, the drugs examined included a competitive antagonist of the strychnine-insensitive glycine binding site (i.e., indole-2-carboxylic acid; I2CA), a noncompetitive allosteric antagonist of the NMDA receptor complex (i.e., [(+)-5-methyl]-10,11-dibenzo [a,d] cyclohepten-5,10-imine maleate; MK-801), and a partial agonist of the strychnine-insensitive glycine binding site (i.e., d-cycloserine) (7,8). Specifically, nimodipine's action would be mediated via inhibition of voltage-gated calcium ion channels, I2CA's action through interference with glycine-stimulated calcium ion conductance, and MK-801's action through noncompetitive antagonism of endogenous glutamate via its binding to an NMDA-associated channel site. Finally, d-cycloserine was expected to stimulate receptor-mediated calcium ion conductance at the strychnine-insensitive glycine binding site. The hypothesis would predict that the effects of nimodipine, I2CA and MK-801 with respect to the

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threshold voltage for seizure production in mice would potentiate those of ethanol, whereas the effect of d-cycloserine would be an antagonistic one.

METHOD

Animals

Experimentally naive, male NIH Swiss mice weighing approximately 20–30 g were used throughout.

Drugs

Indole-2-carboxylic acid (I2CA) and nimodipine were purchased from the Sigma Chemical Co. (St. Louis, MO). d-Cycloserine was purchased from the Aldrich Chemical Co. (Milwaukee, WI). MK-801 was purchased from Research Biochemicals Inc. (Natick, MA). Absolute ethanol (USP 200 proof) was obtained from the Florida Distillers Company (Alfred, FL). d-Cycloserine, MK-801 and absolute ethanol were dissolved in distilled, deionized water. I2CA and nimodipine were suspended in 10% and 5% Tween 80, respectively, via sonication. All drugs and vehicles were prepared freshly on the day of each experiment; they were injected intraperitoneally (IP) in a volume of 0.01 ml/g of body weight.

Incremental Electroconvulsive Shock (IECS) Procedure

In the IECS procedure, a Hittman electroconvulsive shock generator (Medcraft model B24-III) was utilized to administer 0.3 seconds of voltage via earclip electrodes. The procedure began with 70 V and was increased in 10 V increments until a full seizure (maximal tonic hindlimb extension) occurred or 170 V was reached. The antiseizure efficacy of ethanol was assessed in groups who received injections of either vehicle or increasing doses of ethanol (i.e., 0.56, 0.75, 1.0, 1.34 and 1.8 g/kg) 20 minutes prior to the IECS procedure. In all experiments, drugs and their vehicles were injected prior to ethanol and its vehicle according to the following preinjection times: 10 min (I2CA and MK-801), 20 min (nimodipine) and 40 min (d-cycloserine). In all experiments, groups of 12 mice were tested in each of the experimental conditions.

Data from each experiment were analyzed with a two-way analysis of variance. All reports of statistical significance were based on a p value of <0.05.

RESULTS

The first series of experiments examined the effects of three different concentrations of nimodipine (10, 18 and 56 mg/kg) on ethanol's ability to antagonize the electrical precipitation of seizures (Fig. 1). Nimodipine (18 and 56 mg/kg) significantly "upshifted" and "left-shifted" the ethanol dose-response relationship. In the 18 and 56 mg/kg nimodipine conditions, analysis revealed significant main effects for nimodipine and ethanol dose. Thus nimodipine potentiated the antiseizure efficacy of ethanol in the IECS paradigm.

The second series of experiments examined the effect of antagonizing the strychnine-insensitive glycine binding site with I2CA (32 and 100 mg/kg) on ethanol's antiseizure efficacy (Fig. 2). I2CA (100 mg/kg) potentiated the antiseizure efficacy of ethanol. At the 100 mg/kg dose, analysis revealed significant main effects for I2CA and ethanol dose.

In view of the ability of I2CA, a competitive anatagonist of glycine, to potentiate ethanol's antiseizure efficacy, a third experiment was conducted to see whether a noncompetitive NMDA

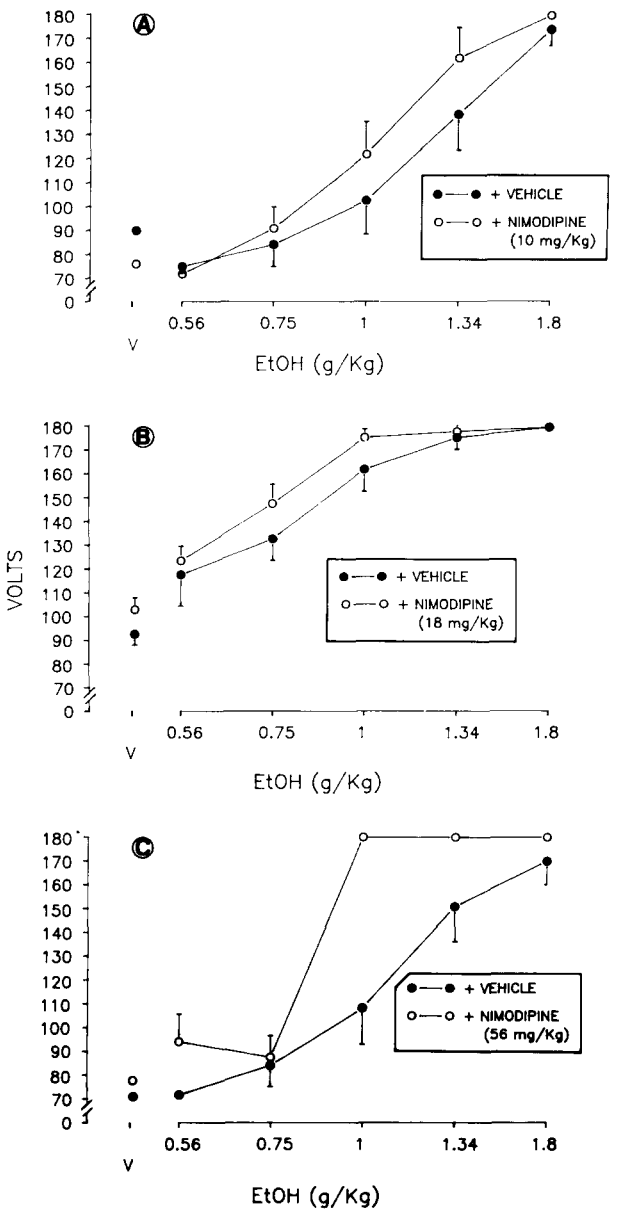

FIG. 1. Figure 1 illustrates the mean voltages (±SEM) for producing seizures in groups of mice (n = 12) injected with either nimodipine (panel A, 10 mg/kg; panel B, 18 mg/kg; panel C, 56 mg/kg; open circles) or the 5% Tween 80 vehicle (closed circles) 20 min prior to an injection of one of the five doses of EtOH (0.56–1.8 g/kg) or its vehicle (distilled water), which was injected 20 min prior to the IECS procedure.

antagonist would act similarly. Therefore, a dose of MK-801 (0.1 mg/kg) that was by itself devoid of antiseizure efficacy was studied for its ability to alter the antiseizure efficacy of ethanol (Fig. 3). Consistent with the prediction, MK-801 increased the efficacy with which ethanol raised the threshold voltage for seizure production. Analysis revealed significant main effects for MK-801 and ethanol dose.

Finally, in view of the ability of I2CA to potentiate ethanol's antiseizure efficacy, we wondered whether d-cycloserine, a glycine agonist at the strychnine-insensitive binding site, might at-

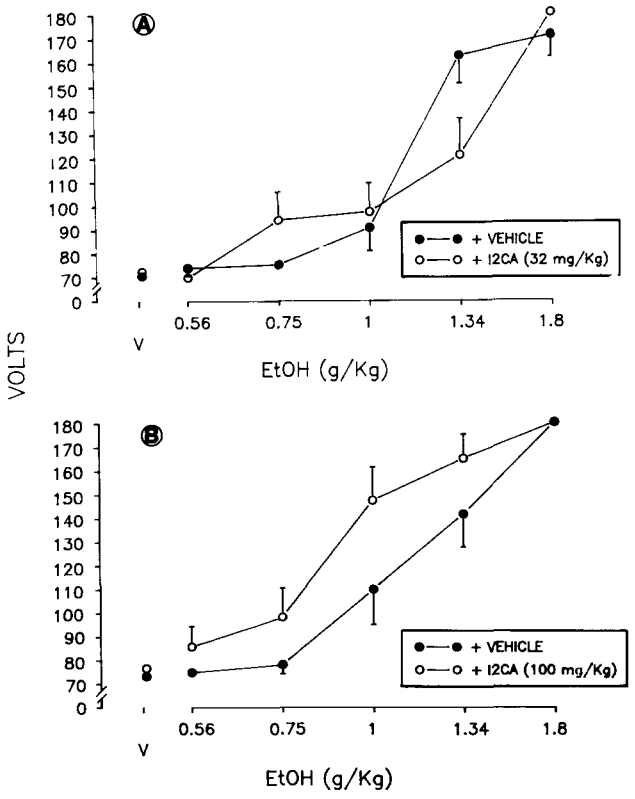

FIG. 2. Figure 2 illustrates the mean voltages (±SEM) for producing seizures in groups of mice (n = 12) injected with either I2CA (panel A, 32 mg/kg; panel B, 100 mg/kg; open circles) or the 10% Tween 80 vehicle (closed circles) 10 min prior to an injection of one of the five doses of EtOH (0.56–1.8 g/kg) or its vehicle (distilled water), which was injected 20 min prior to the IECS procedure.

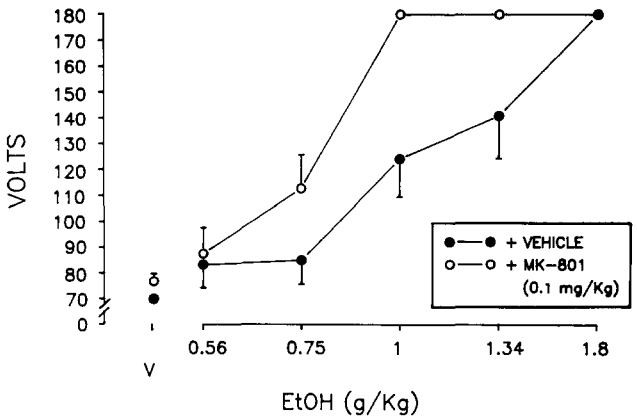

FIG. 3. Figure 3 illustrates the mean voltages (±SEM) for producing seizures in groups of mice (n = 12) injected with either MK-801 (0.1 mg/kg; open circles) or the distilled water vehicle (closed circles) 10 min prior to an injection of one of the five doses of EtOH (0.56–1.8 g/kg) or its vehicle (distilled water), which was injected 20 min prior to the IECS procedure.

tenuate ethanol's antiseizure efficacy. According to this view, d-cycloserine would antagonize ethanol's ability to interfere with NMDA-stimulated calcium ion conductance. There are data to suggest that ethanol interferes with NMDA-mediated conductance via its interaction with the strychnine-insensitive glycine binding (6). In this series of experiments, doses of 10.0, 32.0, and 100 mg/kg d-cycloserine neither antagonized nor potentiated ethanol's antiseizure efficacy (Fig. 4). However, the 320 mg/kg dose of d-cycloserine potentiated ethanol's antiseizure efficacy, as revealed by a significant main effect for d-cycloserine as well as for ethanol.

DISCUSSION

The current series of experiments provide supportive evidence in favor of the hypothesis that, in the intact animal, ethanol's ability to interfere with voltage-gated and receptor-gated calcium ion conductance contributes to its antiseizure efficacy. Presumably, the mechanisms resulting in the antiseizure efficacy of ethanol in mice are similar to those which are responsible for the subjective experience of acute intoxication in the nontolerant human. As per the predictions based on this hypothesis, nimodipine, I2CA and MK-801 were able to potentiate ethanol's antiseizure efficacy. There are data on intact mice showing that nimodipine and verapamil, a calcium-channel antagonist of the phenylalkylamine class, potentiated both the ataxic and hypothermic effects of ethanol, as reflected in a worsening of rotorod performance and reduction in core body temperature (9,10). This is the first demonstration, however, that interference with receptor-gated calcium ion conductance in the intact animal can have effects that potentiate those of ethanol.

Contrary to our initial expectation, in the IECS paradigm, d-cycloserine was not able to antagonize the antiseizure efficacy of ethanol. The three lowest doses of d-cycloserine (10.0, 32.0 and 100 mg/kg) which were explored did not alter the ethanol dose-response curve (Fig. 4). Interestingly, the highest d-cycloserine dose (320 mg/kg) potentiated ethanol in the IECS paradigm. d-Cycloserine is a partial agonist; in radioreceptor binding assays, increasing concentrations of d-cycloserine (e.g., approx. 10^{-5} M and above) antagonized glycine's ability to maximally stimulate ^{3}H-TCP binding, a phencyclidine (PCP) analogue (7). Moreover, the maximal stimulation of ^{3}H-TCP binding that could be achieved with d-cycloserine was only about 40 to 50 percent of the maximal stimulation attainable with glycine. Thus, in the strain of mouse used in our experiments, there is no reason to assume a functional deficiency in the neurotransmitter/neuromodulator pool of glycine. It may be that an effect of d-cycloserine was only observed at concentrations high enough to antagonize endogenous neurotransmitter/neuromodulator pools of glycine in the brain.

In summary, the data obtained in this study support the hypothesis that at least some of ethanol's effects in intact animals may involve interference with calcium ion conductance through voltage-gated and receptor-gated mechanisms. An elucidation of pharmacologically specific effects of ethanol in a concentration range associated with acute intoxication in the nontolerant human should stimulate new avenues for therapeutic intervention in the treatment of alcohol-related disorders.

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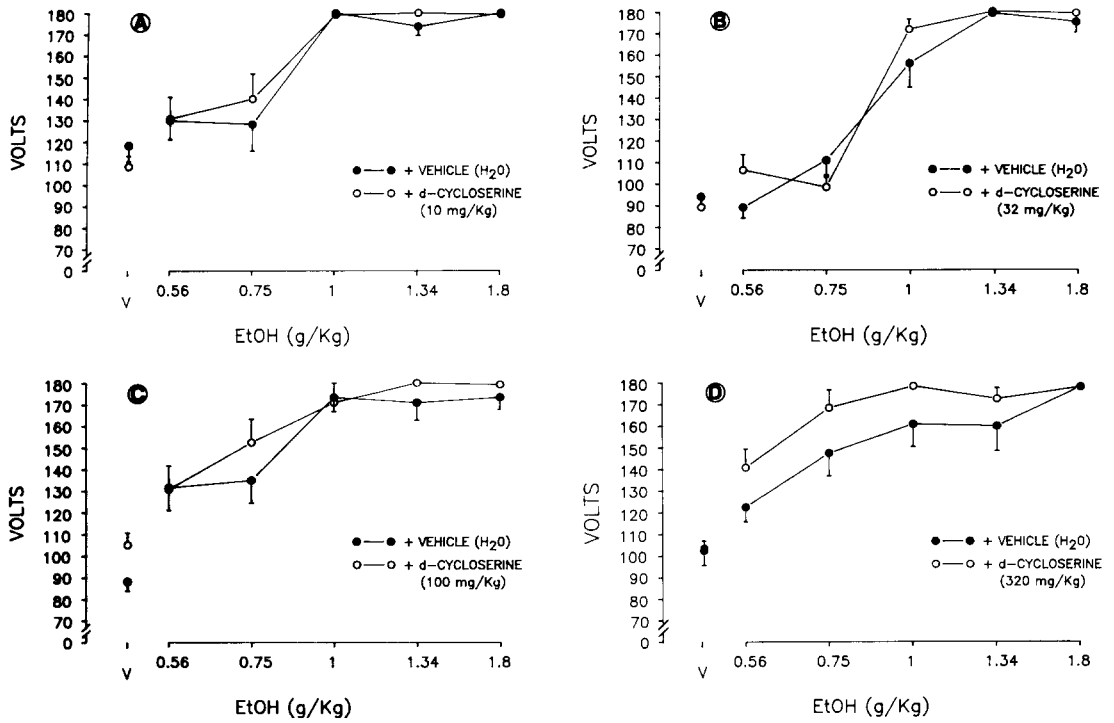

FIG. 4. Figure 4 illustrates the mean voltages (±SEM) for producing seizures in groups of mice (n = 12) injected with either d-cycloserine (panel A, 10 mg/kg; panel B, 32 mg/kg; panel C, 100 mg/kg; panel D, 320 mg/kg; open circles) or the distilled water vehicle (closed circles) 40 min prior to an injection of one of the five doses of EtOH (0.56–1.8 g/kg) or its vehicle, which was injected 20 min prior to the IECS procedure.

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