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BRIEF COMMUNICATION

Glycinergic Interventions Potentiate the Ability of MK 801 to Raise the Threshold Voltage for Tonic Hindlimb Extension in Mice

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NORRIS, D. O., J. MASTROPAOLO, D. A. O'CONNOR, M. R. NOVITZKI AND S. I. DEUTSCH. *Glycinergic interventions potentiate the ability of MK 801 to raise the threshold voltage for tonic hindlimb extension in mice.* PHARMACOL BIOCHEM BEHAV 43(2) 609–612, 1992.—Milacemide, an acylated prodrug of glycine, was able to increase the efficacy with which [+]-5-methyl-10,11-dihydro-5*h*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate (MK 801) antagonized the electrical precipitation of seizures in mice. The mechanism of milacemide's potentiation of MK 801's antiseizure efficacy in intact mice is unclear; however, a glycine agonist selective for the strychnine-insensitive site on the NMDA receptor complex was also able to potentiate MK 801. The exciting possibility exists that an exogenous glycinergic intervention can potentiate NMDA-mediated neural transmission in intact animals.

Glycine Milacemide MK 801 NMDA receptor complex I2CA D-Cycloserine

GLYCINE can potentiate glutamate's ability to stimulate calcium ion conductance through the NMDA receptor complex. Moreover, there are data showing that the presence of glycine is essential to the ability of glutamate to stimulate calcium ion conductance (13). Thus, glycine is a coagonist whose presence is necessary for channel opening. This neuromodulatory action of glycine is mediated by the strychnine-insensitive binding site on the NMDA receptor complex. The binding of open-channel blockers [e.g.,[^{3}H]TCP and [^{3}H][+]-5-methyl-10,11-dihydro-5*h*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate (MK 801)] to well-washed membranes is thought to be a reflection of the activated state of the NMDA receptor complex. In in vitro studies, glycine increases the binding of [^{3}H]MK 801 above the maximal levels achieved with stimulation by glutamate alone (9,16,17). The "glycine-dependent" binding of open-channel blockers is not due to an increase in the density of these channel sites but rather may reflect an increase in the accessibility of these sites to channel ligands and/or an increase in the affinity with which these channel sites bind ligands (16,17,22).

Stimulation of the strychnine-insensitive glycine binding site has been proposed as a therapeutic intervention in neuropsychiatric disorders whose pathophysiology may involve diminished NMDA-mediated neural transmission (5,20). In view of the wide distribution of glycine, it may seem unlikely that exogenous administration of a glycine agonist would contribute to enhancement of glutamatergic transmission (12). Glycine, however, may not be the sole naturally occurring endogenous ligand for the strychnine-insensitive site. Kynurenic acid, a tryptophan metabolite, also binds to the strychnine-site as an antagonist (14).

The present experiments explored the ability of two glycinergic interventions to potentiate NMDA-mediated neural transmission in intact mice. Previous studies in intact animals have shown that glycine or D-serine, a glycine agonist at the strychnine-insensitive site, potentiated MK 801's anticonvul-

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sant action in a maximal electroconvulsive shock paradigm (15), as well as the ability of racemic NMDLA to produce tonic seizures (21), and NMDA-stimulated cerebellar cyclic guanosine monophosphate (cGMP) production (2,23). Whereas glycine may potentiate MK 801's anticonvulsant action, it is limited as a clinically meaningful intervention due to its poor penetration across the blood-brain barrier. Milacemide, a lipophilic prodrug of glycine, however, readily crosses the blood-brain barrier and provides an exogenous source of glycine subsequent to its deacylation by monoamine oxidase B (1,8).

The present study examined the influence of milacemide on the ability of MK 801 to raise the threshold voltage for the precipitation of tonic hindlimb extension in intact mice using an incremental electroconvulsive shock paradigm (7). Based upon in vitro data, the efficacy with which MK 801 antagonizes the electrical precipitation of tonic hindlimb extension may reflect the "activated" state or open configuration of the NMDA receptor complex in the intact mouse. According to this view, an increase in the antiseizure potency of MK 801 on a mg/kg basis results from its increased binding to a specific site within the channel domain. In the present series of experiments, alterations in the antiseizure efficacy of MK 801 are used as an index of NMDA-mediated neural transmission and the activated state of the ionophore in the intact animal. Thus, an increase in the antiseizure efficacy of MK 801 is interpreted to indicate increased channel activation and glutamatergic transmission, whereas a decrease indicates diminished activation and transmission.

The mechanism of milacemide's potentiation of MK 801's antiseizure efficacy in intact mice could involve either stimulation of the strychnine-insensitive site or the glycine-associated chloride ionophore, which is sensitive to inhibition by strychnine, or a combination of the two. Therefore, we studied the ability of milacemide to alter MK 801's antiseizure efficacy in the presence of a specific competitive antagonist of the strychnine-insensitive site, that is, indole-2-carboxylic acid (I2CA) (10). To show that an exogenous glycinergic intervention can influence NMDA-mediated neural transmission in the intact animal, the effect of D-cycloserine, a partial agonist of the strychnine-insensitive glycine receptor (11) that does not interact with the glycine-associated chloride ionophore, was examined on MK 801's ability to antagonize the electrical precipitation of tonic hindlimb extension.

METHOD

Subjects

Experimentally naive, male NIH inbred mice weighing approximately 25 g were used throughout all experiments.

Drugs

Milacemide was obtained from Searle Pharmaceutical, Inc. (Skokie, IL) MK 801 was purchased from Research Biochemicals, Inc. (Natick, MA). D-Cycloserine was purchased from Aldrich Chemical Co. (Milwaukee, WI). Indole-2-carboxylic acid was purchased from Sigma Chemical Co. (St. Louis, MO). Milacemide, MK 801, and D-cycloserine were dissolved in distilled, deionized water. I2CA was suspended in 10% Tween-80 via sonication. All drugs and vehicles were prepared on each day of the experiment. They were injected IP in a volume of 0.01 ml/g of body weight. Milacemide, D-cycloserine, and I2CA were injected 30, 30, and 35 min, respectively, prior to MK 801 or its vehicle. MK 801 (in doses of 0.18, 0.32, 0.56, or 1.0 mg/kg) or its vehicle was injected 30 min prior to testing with the incremental electroconvulsive shock procedure.

Incremental Electroconvulsive Shock (IECS) Procedure

In the IECS procedure, a Hittman electroconvulsive shock generator (Medcraft model B24-III) was utilized to administer 0.3 s of voltage via earclip electrodes. To determine threshold for the precipitation of tonic hindlimb extension voltages, the procedure began with 70 V and was increased in 10-V increments every 2 s until maximal tonic hindlimb extension occurred or 170 V was reached. A voltage of 180 was recorded for animals that did not seize or show tonic hindlimb extension.

Analysis

In all experiments, groups of at least 12 mice were tested in each of the experimental conditions. Data from each experiment were analyzed with a two-way analysis of variance (ANOVA). All reports of statistical significance were based upon a p value of <0.01.

RESULTS

In Experiment 1, there was a significant main effect for dose of MK 801, that is, both animals pretreated with vehicle and milacemide (320 mg/kg) showed a dose-related increase in seizure threshold (see Fig. 1). ANOVA also revealed a significant main effect for pretreatment, that is, an increase in the MK 801 dose-response effect for animals pretreated with milacemide. Seizure thresholds of animals receiving milacemide and vehicle, however, did not differ from seizure thresholds of those receiving vehicle and vehicle. Pretreatment with the glycine prodrug milacemide may have stimulated the strych-

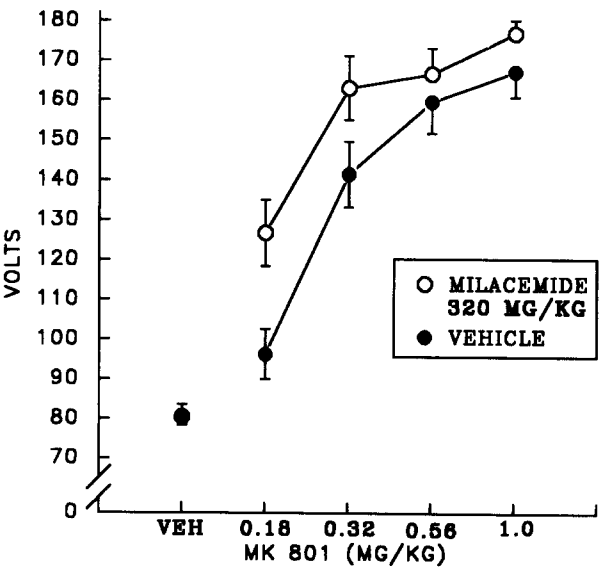

FIG. 1. Mean threshold voltages for tonic hindlimb extension following IP injection of MK 801 (0.18, 0.32, 0.56, or 1.0 mg/kg) or its vehicle. Mice were pretreated with either 320 mg/kg milacemide (○) or its vehicle (●) 30 min prior to administration of MK 801. Subjects receiving vehicle followed by vehicle did not differ from subjects receiving vehicle followed by milacemide.

nine-insensitive site, thereby activating the NMDA receptor complex.

The effect of I2CA pretreatment (100 mg/kg) on the ability of milacemide (320 mg/kg) to potentiate MK 801's antiseizure efficacy was studied. In this experiment, neither I2CA alone nor milacemide alone had a significant effect on the threshold voltage for seizure production. Interestingly, pretreatment with I2CA resulted in milacemide having a significant antiseizure effect even in the absence of MK 801. Furthermore, I2CA pretreatment significantly potentiated MK 801's (0.32 mg/kg) antiseizure efficacy. Finally, I2CA pretreatment did not reduce the ability of milacemide to potentiate MK 801's antiseizure efficacy. These data did not show that milacemide's potentiation of MK 801's antiseizure efficacy was due to its action at the strychnine-insensitive site, subsequent to its enzymatic deacylation.

The final experiment was conducted to show that an exogenous glycinergic intervention that is specific for the strychnine-insensitive site on the NMDA receptor complex can influence the antiseizure efficacy of MK 801 in intact mice. In this experiment, as shown in Fig. 2, neither 320 mg/kg D-cycloserine (D-V) nor 0.32 mg/kg MK 801 (V-MK) alone significantly altered the threshold voltage for seizure production. This dose of D-cycloserine that was ineffective by itself, however, did significantly potentiate MK 801 (D-MK). Thus, an exogenous glycinergic intervention specific for the NMDA receptor complex can influence glutamatergic transmission.

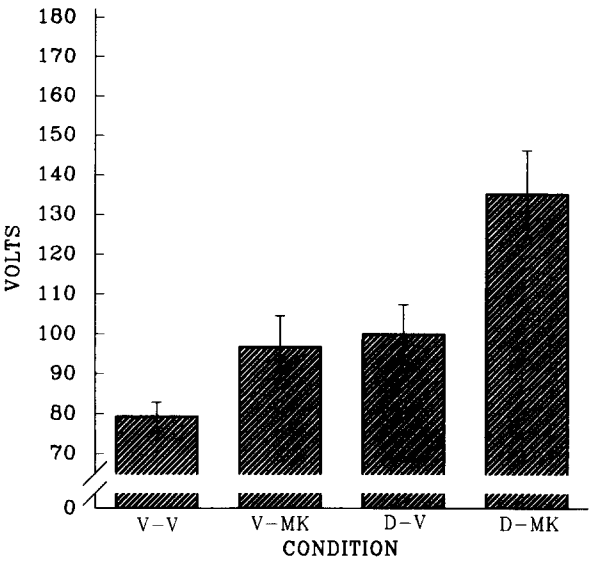

FIG. 2. Mean threshold voltages for tonic hindlimb extension following IP injection of 0.32 mg/kg MK 801 (V-MK, D-MK) or its vehicle (V-V, D-V). Mice were pretreated 30 min prior to MK 801 injections with either D-cycloserine (320 mg/kg) or its vehicle.

DISCUSSION

Deficient NMDA-mediated neural transmission has been implicated in the pathophysiology of schizophrenia, Alzheimer's disease, and acute ethanol intoxication (3–6). Stimulation of the strychnine-insensitive glycine binding site on the NMDA receptor complex has been proposed as a means of potentiating deficient glutamatergic transmission (5,18,19). Unfortunately, the development of therapeutic glycinergic interventions has been delayed because of the paucity of compounds able to cross the blood–brain barrier. Also, glycine is a widely distributed compound whose synthesis can be accomplished in situ and, thus, the strychnine-insensitive site may be maximally stimulated by endogenous glycine. Of course, the neurotransmitter/neuromodulator pool of glycine may be distinct from other metabolic pools.

In the current study, milacemide, an exogenous glycinergic intervention, potentiated the antiseizure efficacy of MK 801 in intact mice. This action of milacemide may be mediated by glycine's agonistic actions at either the strychnine-sensitive glycine receptor in the brainstem or strychnine-insensitive site located on the NMDA receptor complex or a combination of the two. Indole-2-carboxylic acid was used to selectively block the action of glycine at the strychnine-insensitive site to see whether this site mediated the action of milacemide. The results of this experiment demonstrate the difficulty in differentiating between the two possible mechanisms of milacemide's potentiation of MK 801 in the intact animal. The interaction of I2CA and milacemide in the absence of MK 801 resulted in an antiseizure effect. This finding could be due to saturation of strychnine-insensitive sites with I2CA, resulting in greater availability of glycine for promotion of chloride ion conductance in the brain stem. This could account for the inability of I2CA to block milacemide's potentiation of MK 801's antiseizure efficacy. The last experiment showed that D-cycloserine, a glycine agonist at the strychnine-insensitive site, was able to potentiate MK 801's antiseizure efficacy. Thus, an exogenous glycinergic intervention can facilitate NMDA-mediated neural transmission in the intact animal. These data support the development of exogenous glycinergic interventions to potentiate NMDA-mediated neural transmission in neuropsychiatric disorders.

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