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The Antiseizure Efficacies of MK-801, Phencyclidine, Ketamine, and Memantine Are Altered Selectively by Stress

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DEUTSCH, S. I., J. MASTROPAOLO, R. L. RIGGS AND R. B. ROSSE. *The antiseizure efficacies of MK-801, phencyclidine, ketamine, and memantine are altered selectively by stress.* PHARMACOL BIOCHEM BEHAV **58**(3) 709–712, 1997.—Adaptive changes in the NMDA receptor complex occur in response to exposure to stress. We have previously shown that the ability of MK-801, an uncompetitive NMDA receptor antagonist, to antagonize electrically precipitated tonic hindlimb extension is reduced 24 h after mice are forced to swim for up to 10 min in cold water. The stress-induced reduction of the antiseizure efficacy of MK-801 stimulated the proposal that mice exposed to swim stress may serve as "an intact animal model" of altered or diminished NMDA-mediated neural transmission. In the current investigation, the dose-dependent abilities for the antagonism of electrically precipitated seizures in mice were determined for MK-801, phencyclidine, ketamine, and memantine. Interestingly, a single session of cold water swim stress reduced the antiseizure efficacies of MK-801 and memantine without affecting phencyclidine and ketamine when tested 24 h later. The data do not suggest that stress results in a simple reduction in the number of activated or open channels, but rather alters their size or charge characteristics. © 1997 Elsevier Science Inc.

NMDA Receptor complex Stress Seizures

MK-801 ([+]-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-IMINE; dizocilpine), an uncompetitive allosteric antagonist of the *N*-methyl-D-aspartate (NMDA) receptor complex, antagonizes electrically precipitated tonic hindlimb extension in mice in a dose-dependent manner (7,8). In a previous report, we hypothesized that the stress-induced reduction in the antiseizure efficacy of MK-801 provided "an intact animal model" of altered or diminished glutamatergic transmission at the level of the NMDA receptor complex (8). Specifically, 24 h after mice were subjected to a single 10-min session of forced swimming in cold water, the ability of MK-801 to antagonize electrically precipitated seizures was reduced. The mechanism of this alteration of glutamatergic transmission in this animal model is not known.

Alterations of the antiseizure efficacy of MK-801 may reflect changes in the number of NMDA-associated ionophores in the activated or open configuration. Alternatively, this reduction in MK-801's antiseizure efficacy 24 h after a single session of cold water swim stress could reflect a stress-induced change(s) in size or charge characteristics of the ionophore, rather than fewer channels in the activated or open configuration. Stress-induced changes in channel characteristics could result from alterations in levels of endogenous modulators (e.g., neuroactive steroids), phosphorylation, or the unique subunit composition of the NMDA receptor (1,3–6).

The current study was undertaken to determine if our stress procedure reduced the antiseizure efficacy of four uncompetitive allosteric NMDA receptor antagonists in a similar manner. Specifically, we studied the dose-dependent abilities of MK-801, phencyclidine (PCP), ketamine, and memantine to antagonize electrically precipitated seizures, and the effect of forcing mice to swim for up to 10 min in cold water on these dose-dependent abilities 24 h later. PCP, ketamine, and memantine are uncompetitive allosteric antagonists of the NMDA

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receptor complex whose major actions are mediated by binding to the same hydrophobic channel domain as MK-801 (2,10). We reasoned that a consistent reduction in the antiseizure efficacy of all four compounds could reflect fewer activated or open channels. In contrast, if the effect of stress was not the same for all four compounds, this would be consistent with changes in size and/or charge characteristics. All of the experiments and experimental procedures were approved by the Animal Studies Subcommittee and Research Committee of our Department of Veterans Affairs Medical Center. Experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

METHOD

Experimental Procedure

Subjects. An outbred strain of experimentally naive, male NIH Swiss mice purchased from the National Cancer Institute was used throughout all experiments. Animals weighed approximately 25–30 g and were housed (five animals/cage) in a temperature-controlled vivarium with a 12-h light/dark cycle with free access to food and water. Animals were transported to the laboratory on the day of the experiment.

Drugs. Ketamine hydrochloride, memantine hydrochloride, (+)-MK-801 hydrogen maleate, and phencyclidine hydrochloride were all purchased from Research Biochemicals Inc. (Natick, MA). All drugs were dissolved in 0.9% saline and solutions were prepared as needed on the day of each experiment. They were injected intraperitoneally in a volume of 0.01 ml/g of body weight. Ketamine, memantine, and phencyclidine were injected 10 min prior to the shock procedure, and MK-801 was injected 30 min prior to the shock procedure. Ketamine and phencyclidine were administered in doses of 1.8, 3.2, 5.6, 10.0, and 18.0 mg/kg and a vehicle dose. MK-801 was administered in doses of 0.1, 0.18, 0.32, 0.56, and 1.0 mg/kg and a vehicle dose. Memantine was administered in doses of 10.0, 18.0, 32.0, and 56.0 mg/kg and a vehicle dose.

Incremental electroconvulsive shock (IECS) procedure. In the IECS procedure, a Hittman electroconvulsive shock generator (Medcraft model B24-III) was utilized to administer 0.2 s of voltage via earclip electrodes. To determine threshold voltages for the precipitation of tonic hindlimb extension, the procedure began with 70 volts and was increased in 10 volt increments every 2 s until the maximal tonic hindlimb extension occurred or 170 volts was reached. A voltage of 180 was recorded for animals that did not seize or show tonic hindlimb extension.

Stress procedure. Mice were forced to swim in cold (6°C) water for up to 10 min, 24 h prior to testing in the incremental electroconvulsive shock procedure.

Data 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 <0.05.

RESULTS

As shown in Fig. 1, there were significant main effects for both MK-801, $F(5, 132) = 10.94$, and stress, $F(1, 132) = 16.47$. The data indicate that MK-801 raised the threshold voltage for seizure elicitation in a dose-dependent fashion. Also, forcing animals to swim in cold water for up to 10 min reduced the ability of MK-801 to antagonize the electrical precipitation of tonic hindlimb extension.

As shown in Fig. 2, there was a significant main effect for PCP, $F(5, 132) = 37.57$. The data indicate that PCP raised the threshold voltage for seizure elicitation in a dose-dependent

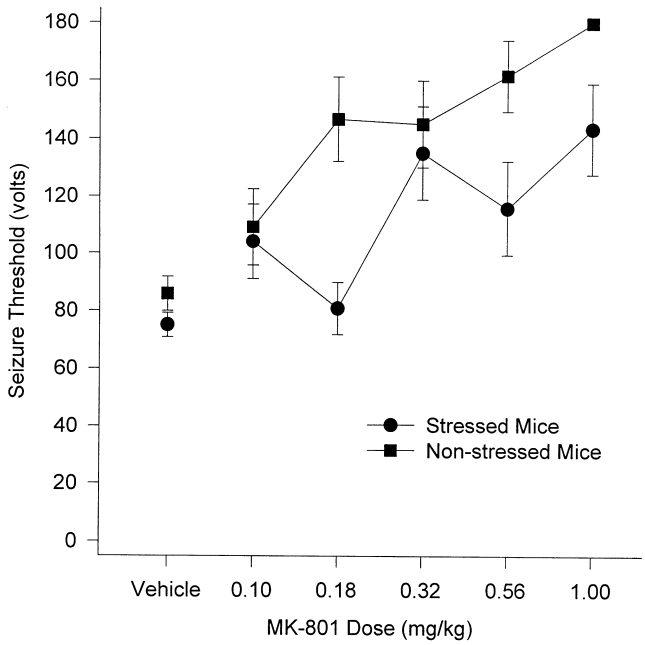

FIG. 1. Mean threshold seizure voltages following intraperitoneal injection of MK-801 (0.1, 0.18, 0.32, 0.56, and 1.0 mg/kg) or its vehicle. Mice were either stressed or not handled 24 h prior to testing.

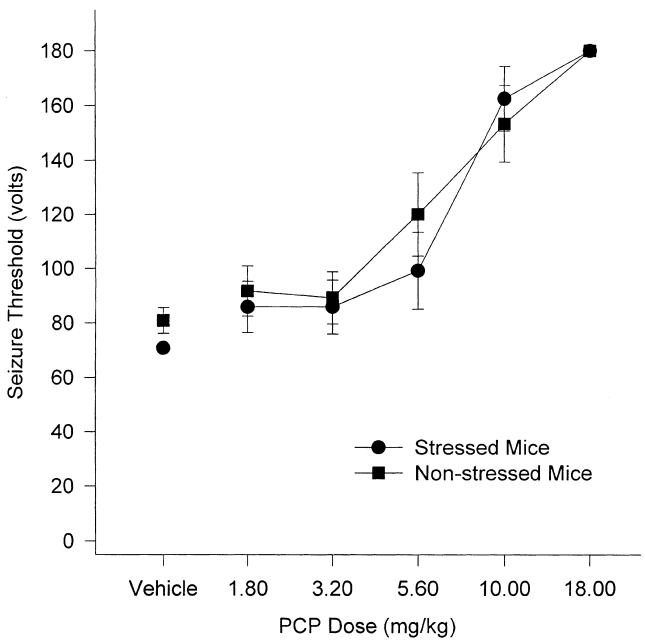

FIG. 2. Mean threshold seizure voltages following intraperitoneal injection of PCP (1.8, 3.2, 5.6, 10.0, and 18.0 mg/kg) or its vehicle. Mice were either stressed or not handled 24 h prior to testing.

fashion. The antiseizure efficacy of PCP was not reduced by forcing animals to swim in cold water for up to 10 min.

As shown in Fig. 3, there was a significant main effect for ketamine, $F(5, 132) = 28.86$. The antiseizure efficacy of ketamine was not reduced by forcing animals to swim in cold water for up to 10 min.

As shown in Fig. 4, there were significant main effects for both memantine, $F(4, 110) = 35.33$, and stress, $F(1, 110) = 17.33$. The data indicate that memantine raised the threshold voltage for seizure elicitation in a dose-dependent fashion. Also, forcing animals to swim in cold water for up to 10 min reduced the ability of memantine to antagonize the electrical precipitation of tonic hindlimb extension.

DISCUSSION

Consistent with our own research, 24 h after rats were forced to swim in ambient temperature water, changes were observed in the potency with which glycine displaced 5,7-^{3}H-dichlorokynurenic acid (5,7-^{3}H-DCKA) from the strychnine-insensitive glycine binding site of the NMDA receptor complex in frontal cortex (9). The increased potency of glycine to competitively inhibit the binding of 5,7-^{3}H-DCKA in stressed tissue was not accompanied by a change in the apparent density of strychnine-insensitive glycine binding sites. Thus, an "adaptation" of the NMDA receptor complex in frontal cortex was interpreted to occur in response to swim stress. Similarly, in view of the failure to observe stress-induced reductions in the antiseizure efficacies of PCP and ketamine, our results suggest that there may not be a simple reduction in the number of activated or open channels, but rather alterations of channel size or charge characteristics. Conceivably, the nature of these functionally relevant alterations of the channel could be "probed" with a series of uncompetitive antagonists. For example, channel entry after stress would be permitted to those molecules sharing shape and charge characteristics with PCP and ketamine, whereas it would be denied to molecules sharing shape and charge characteristics with MK-801 and memantine.

The four compounds studied in this investigation are relatively specific uncompetitive NMDA receptor antagonists; however, their complete spectrum of pharmacological actions does not result exclusively from binding to the hydrophobic domain of the NMDA-associated ionophore. In fact, four parallel lines did not describe the dose–response relations for their antagonism of electrically precipitated seizures, consistent with the existence of multiple targets of action. Thus, there is a possibility that the ability of stress to reduce the antiseizure properties of MK-801 and memantine is unrelated to channel alterations.

The ability of various uncompetitive antagonists to block L-glutamate-evoked currents (e.g., MK-801, PCP, ketamine and N-allylnormetazocine) was shown to vary depending upon the specific subunit compositions of heteromeric receptors expressed in *Xenopus* oocytes (10). Changes in the relative genomic expression of specific subunits in response to stress (e.g., increased expression of the NMDAR2C subunit) could preferentially alter the sensitivity to one uncompetitive antagonist (e.g., decreased sensitivity to MK-801) (3–5). Moreover, functional properties of the channel can be altered rapidly via synthesis and release of endogenous modulators (e.g., D-serine, kynurenic acid, and polyamines), and phosphorylation of intracytoplasmic domains. The current data do not allow us to select which of these possible mechanism(s) accounts for the stress-induced alteration of the antiseizure properties of MK-801 and memantine.

In conclusion, the data may also be relevant to the development and selection of uncompetitive antagonists for the treatment of seizure disorders in the context of a stressful precipitant.

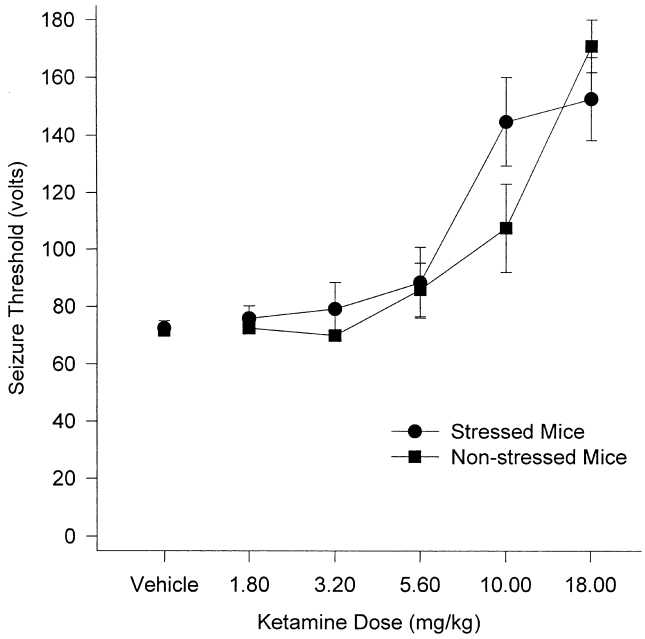

FIG. 3. Mean threshold seizure voltages following intraperitoneal injection of ketamine (1.8, 3.2, 5.6, 10.0, and 18.0 mg/kg) or its vehicle. Mice were either stressed or not handled 24 h prior to testing.

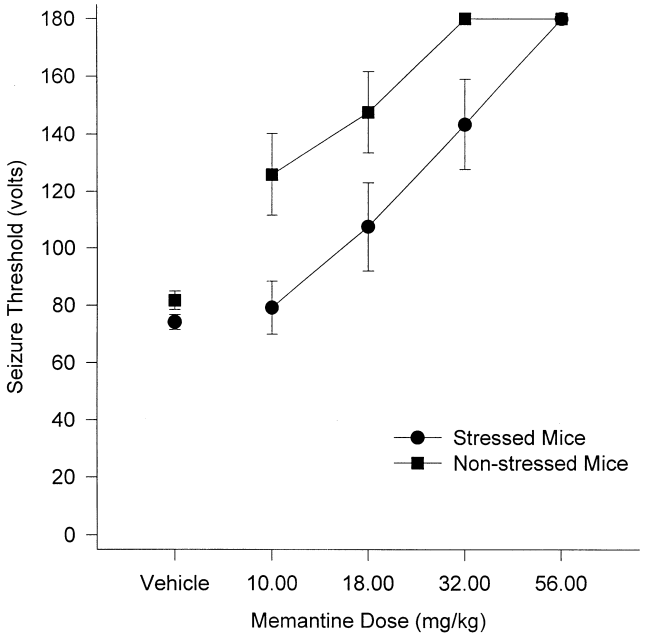

FIG. 4. Mean threshold seizure voltages following intraperitoneal injection of memantine (10.0, 18.0, 32.0, and 56.0 mg/kg) or its vehicle. Mice were either stressed or not handled 24 h prior to testing.

REFERENCES

1. Akinci, M. K.; Johnston, G. A. R.: Sex differences in acute swim stress-induced changes in the binding of MK-801 to the NMDA subclass of glutamate receptors in mouse forebrain. J. Neurochem. 61:2290–2293; 1993.
2. Berger, W.; Deckert, J.; Hartmann, J.; Krotzer, C.; Kornhuber, J.; Ransmayr, G.; Heinsen, H.; Beckmann, H.; Riederer, P.: Memantine inhibits ^{3}H-MK-801 binding to human hippocampal NMDA receptors. Neuroreport 5:1237–1240; 1994.
3. Bresink, I.; Danysz, W.; Parsons, C. G.; Mutschler, E.: Different binding affinities of NMDA receptor channel blockers in various brain regions—Indication of NMDA receptor heterogeneity. Neuropharmacology 34:533–540; 1995.
4. Ferrer-Montiel, A. V.; Sun, W.; Montal, M.: Molecular design of the *N*-methyl-D-aspartate receptor binding site for phencyclidine and dizolcipine. Proc. Natl. Acad. Sci. USA 92:8021–8025; 1995.
5. Ferrer-Montiel, A. V.; Montal, M.: Pentameric subunit stoichiometry of a neuronal glutamate receptor. Proc Natl Acad Sci USA 93: 2741-2744; 1996.
6. Lynch, D. R.; Anegawa, N. J.; Verdoorn, T.; Pritchett, D. B.: *N*-methyl-D-aspartate receptors: Different subunit requirements for binding of glutamate antagonists, glycine antagonists, and channel blocking agents. Mol. Pharmacol. 45:540–545; 1994.
7. Norris, D. O.; Mastropaolo, J.; O'Connor, D. A.; Novitzki, M.; Deutsch, S. I.: Glycinergic interventions potentiate the ability of MK-801 to raise the threshold voltage for tonic hindlimb extension in mice. Pharmacol. Biochem. Behav. 43:609–612; 1992.
8. Norris, D. O.; Mastropaolo, J.; O'Connor, D. A.; Cohen, J. M.; Deutsch, S. I.: A glycinergic intervention potentiates the antiseizure efficacies of MK-801, flurazepam, and carbamazepine. Neurochem. Res. 19:161–165; 1994.
9. Nowak, G.; Redmond, A.; McNamara, M.; Paul, I. A.: Swim stress increases the potency of glycine at the *N*-methyl-D-aspartate receptor complex. J. Neurochem. 64:925–927; 1995.
10. Yamakura, T.; Mori, H.; Masaki, H.; Shimoji, K.; Mishina, M.: Different sensitivities of NMDA receptor channel subtypes to noncompetitive antagonists. Neuroreport 4:687–690; 1993.