

## DIZOCILPINE, KETAMINE AND ETHANOL REVERSE NMDA-INDUCED EEG CHANGES AND CONVULSIONS IN RATS AND MICE

AVADHESH C. SHARMA, SANJAY N. THORAT,  
USHA NAYAR\* AND SHRINIVAS K. KULKARNI\*\*

Pharmacology Division,  
Department of Pharmaceutical Sciences,  
Panjab University, Chandigarh - 160 014

and

\*Department of Physiology,  
All India Institute of Medical Sciences,  
Ansari Nagar, New Delhi - 110 029

( Received on January 7, 1991 )

**Abstract :** Electroencephalographic (EEG) activity in neocortex of rats following intracerebroventricular (icv) administration of NMDA (0.25-2 nmol/10  $\mu$ l) and its modification by noncompetitive NMDA-receptor antagonists, dizocilpine (MK-801) (0.025-0.1 mg/kg, ip) and ketamine (10-50 mg/kg, ip) was recorded at 0, 0.5, 4, 8 and 24 hr with chronically implanted electrodes. NMDA (0.25 and 1 nmol) showed longer lasting decrease in frequency in cortical neurons while 2 nmol produced convulsions and death. Administration of MK 801 (0.05 mg/kg) and ketamine (50 mg/kg) prior to NMDA offered protection in 40% of animals against NMDA-induced convulsions and blocked NMDA-induced long term influence. However, ketamine and MK 801 showed an increase in percent amplitude and also had long lasting effects *per se*. In conscious mice, NMDA (0.5-10 nmol/ $\mu$ l icv) induced dose dependent convulsions. Both MK 801 and ketamine showed potent anticonvulsant effect. Ethanol (0.5-2g/kg, ip) also offered significant protection against NMDA-induced convulsions. MK 801 (0.1 mg/kg) when administered concurrently with ethanol (0.5 g/kg) exhibited synergistic anticonvulsant effect. The EEG study in rats and effect of NMDA in conscious mice provide a direct evidence for the role of NMDA-receptor system in convulsions and in anticonvulsant action of ethanol.

**Key words :** NMDA      dizocilpine      ketamine      ethanol      EEG      convulsions

### INTRODUCTION

Excitatory amino acid (EAA) neurotransmitters, glutamate and aspartate, participate in normal synaptic transmission in brain through a well characterized N-Methyl D-aspartate (NMDA)-receptor system (1, 2). Electrophysiological and radio-ligand binding studies employing selective agonists (e.g. L [ $H^3$ ] glutamate) and antagonist (AP5) suggest widespread distribution of NMDA-receptor(s) in neocortical and hippocampal brain areas which are well in association with motor and memory functions (3, 4, 5, 6). Recent studies demonstrated that activation of NMDA-receptor complex caused the induction

of long term potentiation (LTP) in CA 1 region of hippocampus and cortex and play important role in synaptic plasticity and memory mechanisms (1, 2, 7). It is reported that NMDA-receptor antagonists, competitive and non-competitive blocked induction of LTP and produce acquisition or attentional deficits in several psychobehavioural studies (8, 9, 10, 11, 12). Further, MK 801 and other NMDA antagonists affect EEG sequence in cortical and hippocampal neurons (13, 14). So, the present study was undertaken to investigate EEG changes in rat cortex due to NMDA and its modification by MK 801 and ketamine to understand longer lasting effect, if any.

\*\*Corresponding Author

Besides the normal neurotransmission, EAA-induced neurotoxicity is very well established (1, 2, 15) and NMDA antagonists have potent anticonvulsant profile (16, 17). Anticonvulsant effect of ethanol, which was proposed to be GABAergic in nature (18), also involved blockade of NMDA-activated cationic channel currents (19, 20). The anticonvulsant effect of ethanol was investigated in conscious mice and its interaction with dizocilpine and ketamine was studied.

## METHODS

Male Wistar albino rats (200-250 g; Central Animal House, AIIMS, New Delhi) and mice (20-25 g; CAH, Panjab University, Chandigarh) were used in the study. The animals were housed and maintained on 12 hr light/dark cycle and fed with food and water *ad lib*.

*EEG studies (rat cortex):* Under pentobarbitone anaesthesia (40 mg/kg, ip) electrodes were implanted in neocortex (2.0 mm posterior to bregma, 2.0 mm lateral to midline and 1.5 mm ventral to surface of skull) of rat brain. A polyethylene cannula was also implanted (2.0 mm posterior to bregma, 2.0 mm lateral to midline and 4 mm ventral to the surface of skull) for icv administration of NMDA, taking all necessary aseptic precautions. The animals were kept individually in cages for 3 days to provide recovery from surgical trauma. Each animal was acclimatized to recording room for 24 hr preceding experiment. EEG recordings were carried out on Grass Model 7D Polygraph (Grass Instruments Co., Quincy, Mass, USA) after 0.5, 4, 8 and 24 hrs of administration of MK 801 and ketamine. In case of NMDA, EEG changes were also recorded immediately after icv administration. In combination studies, MK 801 and ketamine were administered 30 min prior to NMDA and recordings were carried out as in NMDA experiments. Data were analysed using one way analysed of variance.

*Centrally administered NMDA- induced convulsions in conscious mice:* Preliminary experiments were performed to find out the icv dose of NMDA (15) in conscious mice that produced convulsions in

all animals. Animals were observed in plexiglass enclosure (28 × 24 × 24 cm) after administration of NMDA for 5 min. Severity of convulsions was assessed using latency for onset of convulsions, % animals exhibiting tonic convulsions and mortality. Data was analysed using *t*-test (delayed onset of convulsions) or by Fisher's exact test.

*Drugs:* The drugs used were, NMDA (Sigma, USA); MK 801 (MSD, England); ketamine (Themis Chemicals Ltd., India); ethanol (Bengal Chemicals, India) and Pentobarbitone sodium (John Baker Inc. USA). All drugs were prepared in distilled water and administered ip, except NMDA which was administered icv (10 µl/rat or 1 µl/mouse).

## RESULTS

*I. Effect of NMDA- receptor ligands on cortical EEG activity of rat brain:* NMDA at lower doses (0.25-1 nmol/rat, icv) showed significant decrease in frequency and the effect was quite evident even after 24 hr of administration (Table I). However, no change in nature of impulses and amplitude was observed (Table II). NMDA (2 nmol) produced severe convulsions and death within few seconds of icv administration. NMDA antagonists, MK 801 and ketamine produced significant decrease in frequency of cortical impulse, though ketamine (50 mg/kg) produced significant increase in frequency even upto 24 hr (Table I). MK 801 showed significant increase in amplitude associated with muscular incoordination (Table II). Ketamine (25 and 50 mg/kg) though showed initial increase in amplitude (0.5 hr) it decreased the same afterwards, which was statistically insignificant (Table II). Administration of MK 801 (0.05 mg/kg) or ketamine (50 mg/kg) followed by NMDA (2 nmol, icv) produced no significant change in cortical EEG activity (Table I and II) but offered protection in 2 out of 5 animals (data not shown in Table).

*II. Effect of various drugs on NMDA- induced convulsions in conscious mice:* Different icv doses of NMDA in mice produced varying degree of convulsions neurotoxicity. NMDA (2 nmol/µl, icv) produced tonic convulsions in 100% animals within

TABLE III : Effect of various doses of NMDA (icv) and of ethanol, MK 801 and ketamine on NMDA (2 nmol)-induced convulsions in mice.

Treatment (mg/kg, ip)	Onset of convulsions S(mean±SE)	% animals showing tonic convulsions	% mortality	(n)
Saline (1 µl, icv)	—	—	—	0/5
NMDA (nmol, icv)				
0.5	20 (1/5)	20	20	1/5
1.0	16.0±1.97	20	20	1/5
2.0	14.30±1.24	100	45	9/20
5.0	13.3±1.55	100	66	4/6
10.0	10.6±1.0	100	60	3/5
NMDA (2 nmol, icv)				
+ ethanol (0.5 g/kg)	13.2 ± 0.87 (5/5)	100	60	3/5
+ ethanol (1.0 g/kg)	14.28 ± 2.64 (2/8)	00	00	0/8
+ ethanol (2.0 g/kg)	—	00	00	0/8 <sup>a</sup>
+ MK 801 (0.05)	12.8 ± 1.25	80	00	0/8 <sup>a</sup>
+ MK 801 (0.1)	12.4 ± 1.31	50	00	0/8 <sup>a</sup>
+ MK 801 (0.25)	40.0 ± 1.14* (2/8)	25	00	0/8 <sup>a</sup>
+ MK 801 (0.5)	—	00	00	0/8 <sup>a</sup>
+ Ketamine (10)	12.8 ± 1.24 (5/8)	62.5	00	0/8 <sup>a</sup>
+ Ketamine (25)	—	00	00	0/9 <sup>a</sup>
+ Ketamine (50)	—	00	00	0/8 <sup>a</sup>

a\*; p < 0.05 as compared to NMDA (2 nmol, icv) group.

TABLE IV : Effect of combined administration of MK 801, ketamine and ethanol in NMDA (2 nmol, icv)- induced convulsions in mice.

Treatment (mg/kg, ip)	Onset of tonic convulsions S(mean±SE)	% animals showing tonic convulsions	% mortality	(n)
NMDA (2 nmol, icv)	14.30 ± 1.24	100	66	4/6
NMDA (2 nmol, icv) + MK 801 (0.5)	11.75 ± 1.67	62.5	12.5	1/8
+ ethanol (0.5 g/kg)	—	00	00	0/10 <sup>a</sup>
+ ketamine (10) + ethanol (0.5 g/kg)	62.6 ± 1.17***	62.5	00	0/8 <sup>a</sup>
+ MK 801 (0.05) + ketamine (10)	56.6 ± 1.09**	62.5	00	0/8 <sup>a</sup>

\*\*p < 0.01 and \*\*\*p < 0.001 as compared to NMDA (2 nmol, icv) treated, group.

a,p < 0.05 as compared by Fischer's exact test.

14.30 ± 1.24 s (Table III). This dose was used in all interaction studies. Ethanol (2 g/kg, ip) completely reversed NMDA-induced tonic convulsions and mortality, whereas in case of lower doses of ethanol

(0.5 and 1.0 g/kg) the number of animals showing onset of convulsions were 2 out of 8 and 5 out of 5, respectively, thereby exhibiting dose dependent action. MK 801 (0.05-0.5 mg/kg) and ketamine (10-50

mg/kg) offered significant protection against NMDA-induced convulsions (Table III). MK 801 (0.05 mg/kg) and ketamine (10 mg/kg), when administered concurrently, produced enhanced anticonvulsant effect as the onset of convulsion was delayed very significantly (Table IV). Similarly, concurrent administration of MK 801 (0.1 mg/kg) or ketamine (10 mg/kg) and ethanol (0.5 g/mg) exhibited synergistic anticonvulsant effect (Table IV), though ethanol (0.5 g/kg) and MK 801 (0.05 mg/kg) have shown partial decrease in % mortality (Table IV).

### DISCUSSION

The present study revealed the anticonvulsant profile of noncompetitive NMDA- receptor antagonists, MK 801 and ketamine in both rats and mice against NMDA- induced epileptogenic neurotoxicity, as reported earlier (15, 16). The phenomenon of induction of long term potentiation by NMDA- receptor agonists, e.g. NMDA, glutamate, has already been described (14) and our results also confirm it, as NMDA (0.25 and 1.0 nmol icv) affect neocortical EEG activity (frequency and amplitude) even upto 24 hrs of its administration. MK 801 (0.025-0.1 mg/kg) also showed significant increase in amplitude even upto 24 hrs where ketamine (25-50 mg/kg) showed increase only at 1/2 hr followed by decrease in amplitude. However, increase in frequency was observed even upto 24 hrs with higher dose of ketamine suggesting that non-competitive NMDA receptor antagonist do possess long term CNS effects. Moreover, whereas MK 801 (0.10 mg/kg) and ketamine (50 mg/kg) caused long term increase in amplitude and frequency, respectively,

NMDA decreased the % in frequency and amplitude suggesting longer lasting, opposite influence of NMDA- receptor ligands (agonist and antagonist). Further, blockade of NMDA- induced longer lasting effect on EEG activity was suggested when MK 801 (0.05 mg/kg) or ketamine were administered prior to NMDA as no significant change in amplitude and frequency was observed (Table I and II) and mortality was also decreased (data not shown), which was observed in the mice also.

The antiepileptic profile of ethanol is well known in several animal studies, however, its mechanism of action is not clear as yet, though several studies suggested the involvement of GABAergic mechanism (18). In the present study ethanol offered potent antiepileptic action against NMDA- induced convulsions in conscious mice which may be due to its ability to antagonise central NMDA- receptor ionophore complex. The non-competitive NMDA- receptor antagonists showed dose-dependent protection against NMDA- induced convulsion, further confirming the earlier reports (15). Interestingly the concurrent administration of MK 801 (0.1 mg/kg) and ethanol enhanced the anticonvulsant effect. Further, ketamine (10 mg/kg) when combined with ethanol significantly delayed the onset of convulsion though % animals showed tonic convulsion remains unaffected. These results tend to suggest that ethanol and NMDA- antagonists produce anticonvulsant effect through similar mechanisms. Recently, it has been reported that NMDA gated cation channels may be a locus of action for neurochemical effects of ethanol (20) where ethanol blocked central NMDA receptor complex.

### REFERENCES

- Collingridge GL, Singer W. EAA pharmacology: excitatory amino acid receptors and synaptic plasticity. *Trends Pharm Sci* 1990; 11 : 290-96.
- Monaghan DT, Bridges RJ, Cotman CW. The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. *Annu Rev Pharmacol Toxicol* 1989; 29 : 365-402.
- Cowburn R, Hardy J, Roberts P, Briggs R. Presynaptic, postsynaptic glutamatergic function in Alzheimer's disease. *Brain Res* 1988; 452: 403-7.
- Fagg GE, L-glutamate, excitatory amino acid receptors and brain function. *Trends Neurosci* 1985; 8 : 207-10.
- Foster AC, Fagg GE. Comparison of L (<sup>3</sup>H) glutamate, D-(<sup>3</sup>H) aspartate, DL-(<sup>3</sup>H) AP5 and (<sup>3</sup>H) NMDA as ligands for NMDA receptors in crude postsynaptic densities from rat brain. *Eur J Pharmacol* 1987; 133 : 291-300.

6. Young AB, Fagg GE. Excitatory amino acid receptors in the brain: membrane binding and receptor autoradiographic approaches. *Trends Pharm Sci* 1990; 11 : 126-33.
7. Collingridge GL, Kehl SJ, McLannan H. Excitatory amino acids in synaptic transmission of Schaffer collateralcommissural pathways in rat hippocampus. *J Physiol* 1983; 334 : 33-46.
8. Butelman ER. A novel NMDA- antagonist, MK 801 impairs performance in a hippocampal dependent spatial learning task. *Pharmacol Biochem Behav* 1989; 34 : 13-16.
9. Sharma AC, Kulkarni SK. Effect of MK 801 and ketamine in short-term memory deficits in passive avoidance stepdown task paradigm in mice. *Meth Find Exptl Clin Pharmacol* 1991; 13: 155-59.
10. Staubli U, Thibault O, Dilorenzo M, Lynch G. Antagonism of NMDA receptors impairs acquisition but not retention of olfactory memory. *Behav Neurosci* 1989; 103 : 54-60.
11. Upchurch M, Wehner JM. Effect of N-methyl-D-aspartate antagonism on spatial learning in mice. *Psychopharmacology* 1990; 100 : 209-14.
12. Venable N, Kelly PH. Effect of NMDA receptor antagonists on passive avoidance learning and retrieval in rats and mice. *Psychopharmacology* 1990; 100 : 215-21.
13. Sagratella S, Benedetti M, Pezzola A, Scotti De Carolis A. Behavioural of electroencephalographic effect of excitatory amino acid antagonists and sigma opiate/phencyclidine-like compounds in rats. *Neuropharmacology* 1989; 28 : 57-61.
14. Whinshaw IQ, Auer RN. Immediate and long lasting effects of MK 801 on motor activity, spatial navigation in a swimming pool and EEG in the rat. *Psychopharmacology* 1989; 98: 500-507.
15. Moreau J, Pieri L, Prud'hon B. Convulsions induced by centrally administered NMDA in mice: Effect of NMDA antagonists, benzodiazepines, minor tranquilizers and anti-convulsants. *Br J Pharmacol* 1989; 98 : 1050-54.
16. Kulkarni SK, Ticku MK. Interaction between GABAergic anticonvulsants and the NMDA receptor antagonist MK 801 against MES-amnd picrotoxin-induced convulsions in rats. *Life Sci* 1989; 44 : 1317-23.
17. Kulkarni SK, Mehta AK, Ticku MK. Comparison of anticonvulsant effect of ethanol against NMDA-, kainic acid- picrotoxin-induced convulsions in rats. *Life Sci* 1990; 46 : 481-87.
18. Lima-Landman MR, Albuquerque EX. Ethanol potentiates and blocks NMDA- activated single channel currents in rat hippocampal pyramidal cells. *FEBS Lett* 1989; 247 : 61-67.
19. Lovinger DM, White G, Weight FF. Ethanol inhibits NMDA- activated ion currents in hippocampal neurons. *Science* 1989; 243 : 1721-24.
20. Gonzales RA. NMDA receptors excites alcohol research *Trends Pharmacol Sci* 1990; 137-39.

6. Young AB, Fagg GE. Excitatory amino acid receptors in the brain: membrane binding and receptor autoradiographic approaches. *Trends Pharm Sci* 1990; 11 : 126-33.
7. Collingridge GL, Kehl SJ, McLannan H. Excitatory amino acids in synaptic transmission of Schaffer collateral-commissural pathways in rat hippocampus. *J Physiol* 1983; 334 : 33-46.
8. Butelman ER. A novel NMDA- antagonist, MK 801 impairs performance in a hippocampal dependent spatial learning task. *Pharmacol Biochem Behav* 1989; 34 : 13-16.
9. Sharma AC, Kulkarni SK. Effect of MK 801 and ketamine in short-term memory deficits in passive avoidance stepdown task paradigm in mice. *Meth Find Exptl Clin Pharmacol* 1991; 13: 155-59.
10. Staubli U, Thibault O, DiIorenzo M, Lynch G. Antagonism of NMDA receptors impairs acquisition but not retention of olfactory memory. *Behav Neurosci* 1989; 103 : 54-60.
11. Upchurch M, Wehner JM. Effect of N-methyl-D-aspartate antagonism on spatial learning in mice. *Psychopharmacology* 1990; 100 : 209-14.
12. Venable N, Kelly PH. Effect of NMDA receptor antagonists on passive avoidance learning and retrieval in rats and mice. *Psychopharmacology* 1990; 100 : 215-21.
13. Sagratella S, Benedetti M, Pezzola A, Scotti De Carolis A. Behavioural of electroencephalographic effect of excitatory amino acid antagonists and sigma opiate/phencyclidine-like compounds in rats. *Neuropharmacology* 1989; 28 : 57-61.
14. Whinshaw IQ, Auer RN. Immediate and long lasting effects of MK 801 on motor activity, spatial navigation in a swimming pool and EEG in the rat. *Psychopharmacology* 1989; 98: 500-507.
15. Moreau J, Pieri L, Prud'hon B. Convulsions induced by centrally administered NMDA in mice: Effect of NMDA antagonists, benzodiazepines, minor tranquilizers and anti-convulsants. *Br J Pharmacol* 1989; 98 : 1050-54.
16. Kulkarni SK, Ticku MK. Interaction between GABAergic anticonvulsants and the NMDA receptor antagonist MK 801 against MES-amnd picrotoxin-induced convulsions in rats. *Life Sci* 1989; 44 : 1317-23.
17. Kulkarni SK, Mehta AK, Ticku MK. Comparison of anticonvulsant effect of ethanol against NMDA-, kainic acid- picrotoxin-induced convulsions in rats. *Life Sci* 1990; 46 : 481-87.
18. Lima-Landman MR, Albuquerque EX. Ethanol potentiates and blocks NMDA- activated single channel currents in rat hippocampal pyramidal cells. *FEBS Lett* 1989; 247 : 61-67.
19. Lovinger DM, White G, Weight FF. Ethanol inhibits NMDA- activated ion currents in hippocampal neurons. *Science* 1989; 243 : 1721-24.
20. Gonzales RA. NMDA receptors excites alcohol research *Trends Pharmacol Sci* 1990; 137-39.