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SHORT COMMUNICATION

N-Methyl-D-Aspartate Prevented Memory Deficits Induced by MK-801 in Mice

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Summary

An interaction between N-methyl-D-aspartate (NMDA) and MK-801 was examined in mice using a modified elevated plus-maze paradigm that allows assessment of the adaptive form of spatial memory. NMDA administered (s.c.) immediately after the acquisition session protected the animals against the amnesia induced by MK-801 given shortly before the retention session. Behavioral performance, expressed as the transfer latency, and therefore spatial memory potency of NMDA plus MK-801 treated animals was comparable with that of both NMDA-treated animals and the controls.

Key words

N-methyl-D-aspartate • MK-801 • Spatial memory • Elevated plus-maze • Mice

The non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 induces dose-dependent impairments of learning and memory (Benvenga and Spaulding 1988, Butelman 1989). To evaluate simply the adaptive form of spatial learning and memory, a modified elevated plus-maze procedure has been developed (Itoh *et al.* 1990). Using this paradigm we have confirmed extensive amnesic effects of MK-801 in mice (Hliňák and Krejčí 1998, 2000, 2002). For example, the ability to recall memory traces on the configuration of the maze was transitorily impaired in animals given MK-801 shortly before the retention session. Memory deficits induced by NMDA receptor antagonists including MK-801 were antagonized by NMDA (Amrick and Bennet 1987, Willetts and Balster 1989, Parada-Turska and Turski 1990). Furthermore, NMDA alone, systemically administered, potentiated cognitive functions in rats (Koek *et al.* 1990, Hliňák and Krejčí 2002).

In this study we investigated whether systemically administered NMDA can prevent amnesia produced by MK-801. The elevated plus-maze paradigm was used. Based on the previous studies (Hliňák and Krejčí 1998, 2000, 2002), the lowest dose of MK-801 (0.15 mg/kg), which was effective in inducing short-term amnesia, was chosen for our experiments.

Experimentally naive female mice (NMRI strain, Konárovice Breeding, Czech Republic), weighing 24-30 g, were housed 10 per cage and kept in a temperature controlled room (20-22 ^{0}C) on a natural day-night period

for at least two weeks before the start of the experiment. Commercial pellet food and water were available *ad libitum*. The experiments were conducted in agreement with the Ethical Direction of State Law 246/1992 (CR).

N-methyl-D-aspartic acid (10, 25 and 50 mg/kg), dissolved in saline, was injected subcutaneously immediately after the acquisition session (AS). MK-801 (0.15 mg/kg), dissolved in saline, was injected intraperitoneally 30 min before the retention session (RS). Both drugs and saline (when appropriate) were given in a volume of 10 ml/kg.

The apparatus and the procedure were described previously (Hliňák and Krejčí 2000). Briefly, the plus-maze consisted of two opposite open arms (30 x 5 cm) and two opposite arms enclosed by 16 cm high walls. The arms were connected by a central platform (5 x 5 cm). The maze was elevated 50 cm from the floor. The animals were randomly assigned to the different experimental and control groups. In the AS, each mouse was placed at the distal end of one open arm facing away from the central platform. The transfer latency (TL), i.e. the time mice took to move from the open arm to the enclosed arm, was recorded. After entering the enclosed arm, the mouse was allowed to move freely in the maze for 10 s. Then, the mouse was returned to its home cage. The RS followed 24 h later. Behavioral testing was conducted between 09:00 and 12:00 h in semi-soundproof room under a natural illumination. The maze was cleaned after each mouse.

The data were evaluated by the Kruskal-Wallis analysis of variance (ANOVA) followed by Dunn´s method and by the Wilcoxon matched-pairs signed ranks test. The criterion for statistical significance was $P<0.05$.

Table 1. The effect of NMDA and MK-801 on the transfer latency (TL) in mice.

Group	Dose mg/kg	N	Acquisition session	Retention session	t	P
Control		10	53.0 ±5.9	20.6±2.8[a]	7.2	<0.0001
MK-801	0.15	10	47.7±4.5	47.2±3.6[b]	0.1	=0.91
NMDA	10	10	47.1±4.7	23.0±2.9[ac]	5.5	=0.0004
NMDA	25	10	53.0±5.7	23.3±2.7[ac]	7.2	<0.0001
NMDA	50	10	49.8±3.9	19.9±1.9[ac]	10.5	<0.0001
NMDA+MK	10+0.15	10	49.4±5.6	36.7±4.7[b]	3.8	=0.004
NMDA+MK	25+0.15	10	47.1±4.7	20.4±2.3[ac]	6.2	=0.0002
NMDA+MK	50+0.15	10	45.6±5.8	20.1±2.5[ac]	4.0	=0.0008
			H=2.31	H=32.8		
			P=0.94	P<0.0001		

Retention session followed 24 h after the acquisition session. NMDA was administered immediately after the acquisition session, MK-801 was given 30 min before the retention session. Transfer latency data (s) are expressed as mean ± S.E.M. values. Statistical analysis, P means probability level of significance (two-tailed): Wilcoxon t-test, [a] retention vs. acquisition session; Kruskal-Wallis ANOVA (H) followed by Dunn´s test, [b] vs. control group, [c] vs. MK-801 group.

The absolute data on the TL are summarized in Table 1. Upon the RS, the overall analysis revealed a significant difference in the TL ($H=32.8$, df 7, $P<0.0001$). As compared to the AS, the controls, NMDA alone (10, 25 and 50 mg/kg) and NMDA plus MK-801 treated mice had a significantly reduced TL; no differences were found among these groups (except for the 10 NMDA + 0.15 MK-801 mg/kg group). The mean TL in MK-801 alone treated animals did not differ from that measured in the AS.

As in the previous studies (Hliňák and Krejčí 1998, 2000, 2002) and the replication here, amnesia occurred in mice given MK-801 shortly before the RS: the dose of 0.15 mg/kg blocked the recall of memory

trace on the spatial configuration of the maze. On the contrary, the animals given NMDA plus MK-801 had the TL significantly reduced and identical to that of the controls, which means that the animals were able to escape rapidly from the unsafe to the safe arms of the maze. This finding can be interpreted as an NMDA-induced protection against the MK-801 elicited amnesia. The same behavioral effect was observed in mice pretreated with the nootropic oxiracetam (Hliňák and Krejčí 2000).

Because the NMDA receptors are required at the time of training and for a few seconds afterwards, but not later (Izquierdo and Medina 1997), our results are in accordance with the idea that hippocampal NMDA receptors participate in the consolidation phase of memory processing (Steele and Morris 1999, Save and Poucet 2000). A rapid and robust storage of spatial information under the influence of NMDA is apparently associated with protein synthesis which is implicated in memory formation (Radulovic *et al.* 1998, Vann *et al.* 2000). The created memory trace seems to be resistant against the disruptive (amnesic) effect of MK-801. In conclusion, the proper mechanism by which NMDA prevents the MK-801 induced amnesia cannot be explained on the basis of the present data. The effect of both compounds when administered simultaneously and immediately after the AS appears of special interest. The simple elegance of the used procedure supports its usefulness in studies of the neurobiology of memory.

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References

AMRICK CL, BENNETT DA: N-methyl-D-aspartate produces discriminative stimuli in rats. *Life Sci* **40**: 585-591, 1987.

BENVENGA MJ, SPAULDING TC: Amnesic effect of the novel anticonvulsant MK-801. *Pharmacol Biochem Behav* **30**: 205-207, 1988.

BUTELMAN ER: A novel NMDA antagonist, MK-801, impairs performance in a hippocampal-dependent spatial learning task. *Pharmacol Biochem Behav* **34**: 13-16, 1989.

HLIŇÁK Z, KREJČÍ I: Concurrent administration of subeffective doses of scopolamine and MK-801 produces a short-term amnesia for the elevated plus-maze in mice. *Behav Brain Res* **91**:83-89, 1998.

HLIŇÁK Z, KREJČÍ I: Oxiracetam prevents the MK-801 induced amnesia for the elevated plus-maze in mice. *Behav Brain Res* **117**: 147-151, 2000.

HLIŇÁK Z, KREJČÍ I: MK-801 induced amnesia for the elevated plus-maze in mice. *Behav Brain Res* **131**: 221-225, 2002.

ITOH J, NABESHIMA T, KAMEYAMA T: Utility of an elevated plus-maze for dissociation of amnesic and behavioral effects of drugs in mice. *Psychopharmacology* **101**: 27-33, 1990.

IZQUIERDO I, MEDINA JH: Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. *Neurobiol Learn Mem* **68**: 285-316, 1997.

KOEK W, WOODS JH, COLPAERT FC: N-methyl-D-aspartate antagonism and phencyclidine-like activity: a drug discrimination analysis. *J Pharmacol Exp Ther* **253**: 1017-1025, 1990.

PARADA-TURSKA J, TURSKI WA: Excitatory amino acid antagonists and memory: effect of drugs acting at N-methyl-D-aspartate receptors in learning and memory. *Neuropharmacology* **29**: 1111-1116, 1990.

RADULOVIC J, KAMMERMEIER J, SPIESS J: Relation between Fos production and classical fear conditioning: effects of novelty, latent inhibition and unconditioned stimulus preexposure. *J Neurosci* **18**: 7452-7461, 1998.

SAVE E, POUCET B: Hippocampal-parietal cortical interactions in spatial cognition. *Hippocampus* **10**: 491-499, 2000.

STEELE RJ, MORRIS RG: Delay-dependent impairment of a matching-to-phase task with chronic and intrahippocampal infusion of the NMDA antagonist D-AP5. *Hippocampus* **9:** 118-136, 1999.

VANN SD, BROWN MW, AGGLETON JP: Fos expression in the rostral thalamic nuclei and associated cortical regions in response to different spatial memory task. *Neuroscience* **101**: 983-991, 2000.

WILLETS J, BALSTER RL: Effects of competitive and non-competitive N-methyl-D-aspartate (NMDA) antagonists in rats trained to disriminate NMDA from saline. *J Pharmacol Exp Ther* **251**: 627-633, 1989.

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