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RAPID COMMUNICATION

# Combined Effects of Ethanol and MK 801 on Locomotor Activity in the Rat

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ROBLEDO, P., W. KANEKO AND C. L. EHLERS. *Combined effects of ethanol and MK 801 on locomotor activity in the rat.* PHARMACOL BIOCHEM BEHAV **39**(2) 513–516, 1991.—The present study examined the effects of ethanol (0.75 g/kg IP) alone and in combination with the noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist MK 801 (0.1 mg/kg SC) on the locomotor activity of rats. Sixteen rats were treated with vehicle plus saline, MK 801 plus saline, vehicle plus ethanol, and MK 801 plus ethanol. Locomotor activity was quantified for a period of 12 hours following drug administration. Ethanol was found to significantly decrease locomotor activity whereas MK 801 significantly increased locomotion during the first 2 hours postdrug. In addition, there was a significant additive interaction between ethanol and MK 801 during this time period. Two to four hours postdrug, MK 801 was observed to significantly decrease locomotion. Four to six hours postdrug, ethanol-treated rats had significantly increased locomotor activity whereas MK 801-treated rats displayed significantly decreased locomotion. No significant interaction was found between ethanol and MK 801 4 to 6 hours postdrug. No significant effects of any of the drugs on locomotor activity were observed from 6 to 12 hours postdrug. These results suggest that ethanol and MK 801 produce a pattern of effects on locomotor activity which depend on the time elapsed following drug administration.

Alcohol NMDA Receptor Locomotion Activity cages

SEVERAL lines of evidence suggest that some of the actions of ethanol involve an interaction with the N-methyl-D-aspartate (NMDA) receptor complex. Recently, it has been shown that ethanol inhibits the synaptic excitation mediated by NMDA receptors in rat hippocampal slices, and that this may contribute to the intoxicating effects of ethanol (15). Further evidence that NMDA receptors may mediate the effects of ethanol comes from studies utilizing the noncompetitive NMDA receptor antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine maleate (MK 801). For example, MK 801 has been found to reduce the seizure score in alcohol withdrawing rats, suggesting that chronic alcohol may supersensitize the NMDA receptor (16). MK 801, apart from being a powerful anticonvulsant and muscle relaxant in and of itself [see (18) for review], has also been shown to potentiate the anticonvulsant (18) and anxiolytic (6) effects of ethanol in rats.

Some of the behavioral effects of MK 801 are known to be similar to "PCP-type" drugs. Increases in locomotion, sniffing, swaying and falling have all been described following MK 801 administration (10). Specific locomotor patterns induced by a low dose of MK 801 (0.5 mg/kg) include hyperactive behaviors such as increases in distance traveled, speed, and clockwise/anticlockwise locomotion, along with decreases in rearing (5).

While the interactions of MK 801 and ethanol on locomotor behavior are not yet described, the effects of ethanol on locomotion are well known. Depending on the dose, ethanol has been shown to produce locomotor stimulation (4,9), depression of locomotor activity (13,14), and biphasic effects [see (17) for review]. The locomotor stimulation observed with low doses of ethanol has been attributed to its actions on catecholaminergic systems (1), particularly the dopaminergic system [(19), see (11) for review]. Similarly, the locomotor stimulation produced by low doses of MK 801 has also been suggested to be mediated by dopaminergic processes (2, 3, 7).

The aim of the present study was to further characterize the locomotor effects of low doses of MK 801 and ethanol, alone and in combination, during the active phase of the rats cycle in order to determine if these two drugs possessed synergistic effects.

## METHOD

Sixteen male Wistar rats (Charles River, USA) weighing between 250 and 300 g were used in this study. The rats were grouped housed under a 12-h on/12-h off light cycle (lights on at 0600 h), and had unrestricted access to rat chow and water. Locomotor activity was quantified in a bank of 16 wire cages (25 × 20 × 36 cm) with two horizontal infrared photocell beams

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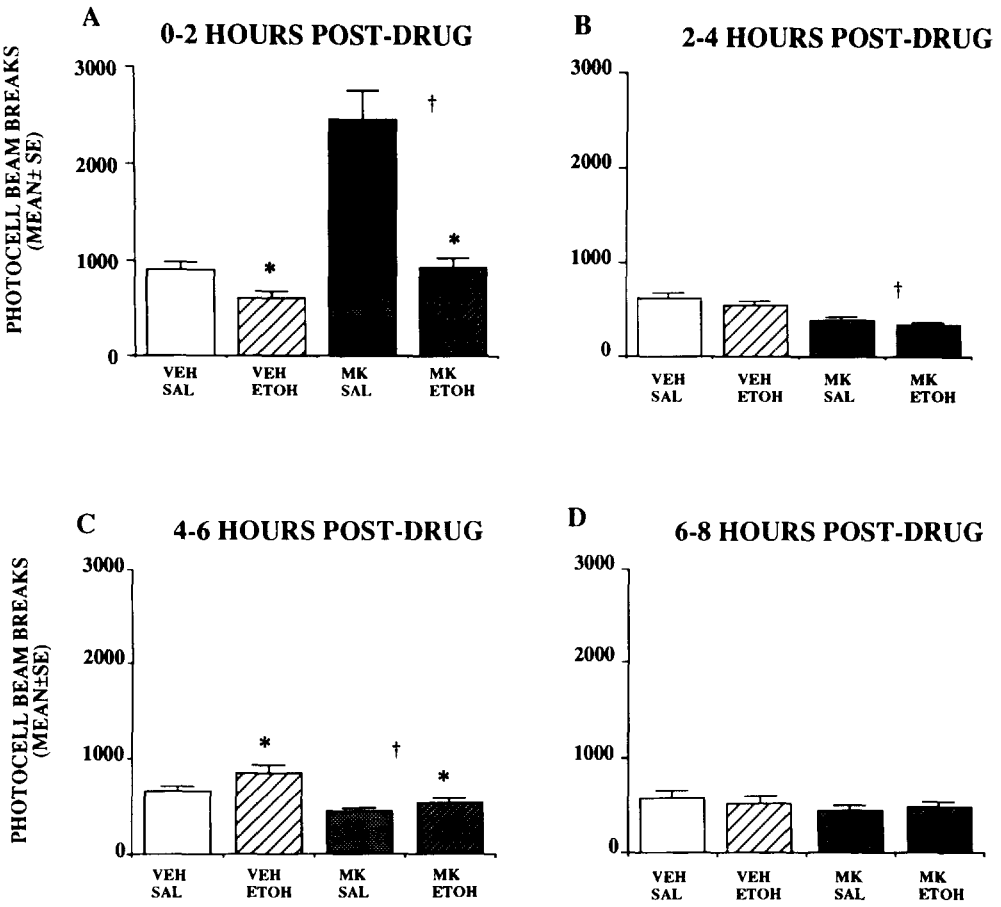


FIG. 1. Locomotor effects as quantified by photocell counts, of ethanol and MK 801 administered alone and in combination over eight hours postdrug testing period. Results are expressed as mean ± S.E.M. Saline (SAL), ethanol (ETOH, 0.75 g/kg IP), MK 801 (MK, 0.1 mg/kg SC), n = 16 for each treatment condition. *Significant main effect of ethanol (ANOVA). †Significant main effect of MK 801 (ANOVA).

located across the long axis of each cage. Photocell interruptions were recorded by a computer in intervals of 10 minutes. Rats were habituated to the activity cages for a 12-h period several days prior to the first test session. Animals were first subcutaneously (SC) injected with either vehicle or MK 801 (0.1 mg/kg; Merck Sharp and Dohme Pharmaceuticals), 15 minutes later they received an intraperitoneal (IP) injection of either saline or 10% ethanol (0.75 g/kg). All animals were tested on four occasions with an interval of 10 days between each test. On the first and second test sessions all animals received a subcutaneous injection of vehicle followed by a intraperitoneal injection of saline, or MK 801 SC followed by saline (IP), on the third and fourth test sessions all animals received vehicle (SC) followed by ethanol (IP), or MK 801 (SC) followed by ethanol (IP). Locomotor activity measurements began 10 minutes after the last injection and were carried out during the active phase of the rats cycle (1800 h to 0600 h). Statistical analysis of the 12-hour locomotor activity data was carried out for intervals of 2 hours each using a two-way within subject analyses of variance (ANOVA) with ethanol as the first factor and MK 801 as the second factor, followed by simple effects post hoc analyses for the ethanol-MK 801 interaction.

## RESULTS

Figure 1A shows that during the first 2 hours postdrug, the administration of ethanol produced decreases in locomotor activity while MK 801 at the dose of 0.1 mg/kg produced increases in locomotion [significant main effect for the presence of MK 801, $F(1,15) = 20.7$, $p<0.001$; significant main effect for the presence of ethanol, $F(1,15) = 58.7$, $p<0.001$]. A significant interaction between ethanol and MK 801 was also observed during this time period, $F(1,15) = 21.2$, $p<0.001$ (Fig. 1A), showing that locomotor activity was decreased more by ethanol when it was administered with MK 801 than with vehicle. As seen in Fig. 1B, 2 to 4 hours postdrug, MK 801 was found to reduce locomotor activity [significant main effect for the presence of MK 801, $F(1,15) = 35.4$, $p<0.001$, Fig. 1B], whereas ethanol did not produce a significant main effect on locomotor activity and there was no interaction between ethanol and MK 801. Four to six hours postdrug, as shown in Fig. 1C, ethanol was found to increase locomotor activity while MK 801 was observed to decrease locomotor activity [significant main effect for the presence of ethanol, $F(1,15) = 15.8$, $p<0.001$; significant main effect for the presence of MK 801, $F(1,15) = 20.4$, $p<0.001$]. No significant interaction was found between these two drugs during this time period. Fig. 1D shows locomotor activity during 6 to 8 hours postdrug where no significant effects of either drug were found. No effects were observed during 8 to 12 hours postdrug period (data not shown).

## DISCUSSION

Biochemical data showing an increase in L-glutamate bind-

ing sites in the brain of rats chronically treated with ethanol have suggested that ethanol may interact with the glutamate receptor complex (15). Glutamate is a major excitatory neurotransmitter in the central nervous system interacting with three different receptor subtypes indentified according to their agonist specificities: kainate, quisqualate and N-methyl-D-aspartate (NMDA) (20). In more recent electrophysiological and biochemical experiments it has been shown that ethanol at low concentrations, selectively inhibits NMDA receptor mediated processes and that the other glutamate receptor subtypes may not be as sensitive to the actions of ethanol (8,12).

In the present study, the noncompetitive NMDA receptor antagonist MK 801 was found to produce a marked increase in locomotion during the first 2 hours postinjection. On the contrary, low doses of ethanol (0.75 g/kg) were found to decrease the locomotor activity during this time period. The literature reports a diversity of effects following ethanol administration depending on the species and the strain of the animals used and the dose of ethanol administered [see (17) for review]. A stimulant effect of ethanol has been found most frequently at very low doses (0.1–0.8 g/kg) in both rats and mice [see (14) for review]. This effect is attributed to the involvement of central catecholaminergic systems (1), and more specifically to the dopaminergic system (11,19). On the other hand, depressant effects of ethanol have been observed in Wistar rats at higher doses (1.0–4.0 g/kg) and seem to be mediated mostly through noradrenergic processes (14). The low doses of ethanol administered in this study (0.75 g/kg) were found to produce decreases in locomotor activity, not increases as might be predicted by the literature. This apparent discrepancy in results may be due to differences in testing methodology, or to the fact that our study was conducted during the dark cycle when rats are more active and thus possibly more sensitive to the depressant effects of ethanol.

The results showing that MK 801 increases locomotion during 0 to 2 hours postdrug are in agreement with previous studies showing locomotor stimulation in rats and in mice following MK 801 administration when the drug is administered during the inactive part of the rats light cycle (3,21). A widely accepted belief is that MK 801 produces locomotor stimulation through blockade of NMDA receptors presumably leading to catecholamine release (3). Recently, this hypothesis has been supported by the findings that MK 801 increases dopamine turnover in the striatum and cortex of rats (7). Some evidence also supports a catecholamine-independent mode of action for MK 801. Carlsson and Svensson (1990) have recently reported that MK 801 can produce marked locomotor stimulation in monoamine-depleted mice in motility cages. These same authors report that in monoamine-depleted rats, MK 801 (0.1 mg/kg) induced behavioral activation in an open field. However, when measured in the motility cages, MK 801 alone failed to stimulate locomotion, although a pronounced enhancement of locomotor activity was observed when it was combined with the α-adrenergic agonist, clonidine.

In the present study, the initial increase in locomotion observed after the administration of MK 801 was followed by a decrease in locomotion starting at 2 hours postdrug and lasting through 6 hours postdrug. Ethanol also showed a biphasic effect on locomotor activity, first decreasing locomotion, then increasing during the 4 to 6 hours postdrug. During the first 2-hour period postdrug, the ethanol and MK 801 interaction was found to be additive in that the decrease in locomotor activity produced by ethanol and the increase produced by MK 801 summed up to aproximately baseline level. The fact that ethanol and MK 801 produced a different pattern of locomotor effects in these dose ranges suggest that they may be acting through two different mechanisms. Ethanol's depressant effects may involve GABAergic (13) or noradrenergic systems (14) while MK 801's stimulant effects may involve dopaminergic or PCP-like processes.

In summary, this study shows that low doses of ethanol produce depressant effects on locomotor activity when administered during the active phase of the rats cycle, whereas low doses of MK 801 produce the opposite effects, e.g., stimulation of locomotor activity. When these two substances are combined they do not show synergistic effects but in fact their effects tend to cancel each other out.

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## REFERENCES

1. Carlsson, A.; Engel, J.; Svensson, T. H. Inhibition of ethanol-induced excitation in mice and rats by α-methyl-p-tyrosine. Psychopharmacologia 26:307–312; 1972.
2. Carlsson, A.; Svensson, A. Interfering with glutamatergic neurotransmission by means of NMDA antagonist administration discloses the locomotor stimulatory potential of other transmitter systems. Pharmacol. Biochem. Behav. 36:45–50; 1990.
3. Clineschmidt, B. V.; Martin, G. E.; Bunting, P. R.; Papp, N. L. Central sympathomimetic activity of (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine (MK-801), a substance with potent anticonvulsant, central sympathomimetic and apparent anxiolytic properties. Drug Dev. Res. 2:135–145; 1982.
4. Cott, J. M.; Carlsson, J. E.; Lindqvist, M. Suppression of ethanol-induced locomotor stimulation by GABA-like drugs. Naunyn Schmeidebergs Arch. Pharmacol. 295:203–209; 1976.
5. Ford, L. M.; Norman, A. B.; Sambert, P. R. The topography of MK-801-induced locomotor patterns in rats. Physiol. Behav. 46: 755–758; 1989.
6. Goldberg, M. E.; Salama, A. I.; Patel, J. B.; Malick, J. B.; Novel non-benzodiazepine anxiolytics. Neuropharmacology 22:1499–1504; 1983.
7. Hiramatsu, M.; Cho, A. K.; Nabeshima, T. Comparison of the behavioral and biochemical effects of the NMDA receptor antagonists, MK-801 and phencyclidine. Eur. J. Pharmacol. 166:359–366; 1989.
8. Hoffman, P. L.; Rabe, C. S.; Grant, K. A.; Valverius, P.; Hudspith, M.; Tabakoff, B. Ethanol and the NMDA receptor. Alcohol 7:229–231; 1990.
9. Kocchar, A.; Erickson, C. K. Central cholinergic correlates of low dose ethanol-induced locomotor stimulation. Alcohol.: Clin. Exp. Res. 10:595–601; 1986.
10. Koeh, W.; Woods, J. H.; Gail, D. W. MK-801, a proposed noncompetitive antagonist of excitatory amino acid neurotransmission, produces phencyclidine-like behavioral effects in pigeons, rats and rhesus monkeys. J. Pharmacol. Exp. Ther. 245:1019–1027; 1988.
11. Lewis, M. J.; June, H. L. Neurobehavioral studies of ethanol reward and activation. Alcohol 7:213–219; 1990.
12. Lovinger, D. M.; White, G.; Weight, F. F. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science 243:1721–1724; 1989.
13. Martz, A.; Deitrich, R. A.; Harris, A. Behavioral evidence for the involvement of γ-aminobutyric acid in the actions of ethanol. Eur. J. Pharmacol. 89:53–62; 1983.
14. Mason, S. T.; Corcoran, M. E.; Fibiger, H. C. Noradrenergic processes involved in the locomotor effects of ethanol. Eur. J. Pharmacol. 54:383–387; 1979.
15. Michaelis, E. K.; Mulvaney, M. J.; Freed, W. J. Effects of acute and chronic ethanol intake on synaptosomal glutamate binding activity. Biochem. Pharmacol. 27:1685–1691; 1978.
16. Morrisett, R. A.; Rezvani, A. H.; Overstreet, D.; Janowsky, D. S.; Wilson, W. A.; Swartzwelder, H. S. MK-801 potently inhibits al-

cohol withdrawal seizures in rats. Eur. J. Pharmacol. 176:103–105; 1989.
17. Pohorecky, L. A. Biphasic actions of ethanol. Biobehav. Rev. 1:231–240; 1977.
18. Shrinivas, K. K.; Ticku, M. K. Interaction between gabaergic anticonvulsants and the NMDA receptor antagonist NK 801 against MES- and picrotoxin-induced convulsions in rats. Life Sci. 44: 1317–1323; 1989.
19. Strombom, U. F.; Liedman, B. Role of dopaminergic neurotransmission in locomotor stimulation by dexamphetamine and ethanol. Psychopharmacology (Berlin) 78:271–276; 1982.
20. Watkins, J. C.; Evans, R. H. Excitatory amino acid transmitters. Annu. Rev. Pharmacol. Toxicol. 21:165–204; 1981.
21. Whishaw, I. Q.; Auer, R. N. Immediate and long-lasting effects of MK-801 on motor activity, spatial navigation in swimming pool and EEG in the rat. Psychopharmacology (Berlin) 98:500–507; 1989.