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Involvement of Dopamine Receptors on Locomotor Stimulation and Sensitization Elicited by the Interaction of Ethanol and Mazindol in Mice

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GEVAERD, M. S. AND R. N. TAKAHASHI. *Involvement of dopamine receptors on locomotor stimulation and sensitization elicited by the interaction of ethanol and mazindol in mice.* PHARMACOL BIOCHEM BEHAV **63**(3) 395–399, 1999.—We have previously observed that the combination of ethanol (EtOH) and the anorectic drug mazindol (MZ) produces more marked effects on behavior than either substance alone. In the present study we examined whether the repeated administration of the drug combination could induce sensitization to its motor activating effects in mice and, if so, whether this response could be affected by dopamine (DA) receptors antagonists. Male Swiss albino mice were treated daily for 7 days with combined EtOH+MZ (1.2 g/kg, 5.0 mg/kg IP), EtOH (1.2 g/kg IP), MZ (5.0 mg/kg IP), or control solution coadministered with the D_1 dopamine antagonist SCH-23390 (0.025 or 0.05 mg/kg IP), the mixed dopamine antagonist haloperidol (0.05 or 0.075 mg/kg IP), or vehicle. After the injections on days 1, 7, and 10, mice were assessed in activity cages at different time intervals. Repeated administration of MZ resulted in an enhancement of its locomotor activating effects, behavioral sensitization. Further, the combined EtOH+MZ treatment also resulted in sensitization to its locomotor effects. Moreover, the development of MZ and EtOH+MZ sensitization was attenuated by both SCH-23390 and haloperidol. These data demonstrate that following repeated MZ or EtOH+MZ exposure mice show locomotor sensitization through DA receptor stimulation. Also, these findings suggest that a major determinant of combined anorectic-alcohol misuse may be the increased stimulating effects produced by such combination. © 1999 Elsevier Science Inc.

Mazindol Ethanol Locomotor activity Sensitization Dopamine Mice

IN Brazil, the consumption of anorectic drugs with catecholaminergic action (diethylpropion, fenproporex, and mazindol) have reached considerable proportions. For example, recent reports have shown that in 1992 the Brazilian consumption rates for these compounds topped 23 metric tons, and that approximately half of this consumption was formulated as nonproprietary prescriptions (18,19). Among the misused anorectics, mazindol (MZ) is a nonphenylethylamine compound known to act primarily by inhibiting dopamine (DA) uptake (7,8,21). Therefore, similarly to cocaine and *d*-amphetamine, MZ increases locomotor activity, reduces food intake, induces circling, stereotypes, and self-administration behavior in animals (11). In addition, several reports have shown that repeated administration of cocaine-like stimulants results in an altered behavioral response to subsequent administration of the drug (1,4,13,14,26). Accordingly, a recent study in our laboratory has shown that repeated MZ administration progressively increases the locomotor response in rats (28).

On the other hand, the combined anorectic-alcohol misuse is also a prevalent problem in Brazil. Indeed, there are anecdotal reports relating the extensive consumption of anorectic drugs by truck drivers in order to stay awake, and an aggra-

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vating factor is that, in some cases, the anorectic is taken with alcohol. Such combination usage may reflect the popular belief that by mixing alcohol and MZ, an enhancement of the stimulatory action of MZ is reached. Thus, our investigation aiming at this interaction confirmed the increased locomotor stimulating effect of the EtOH+MZ combination, and that this response was not simply additive effects of separate action of EtOH and MZ (6).

The enhancement of sensitivity, following repeated exposure to psychostimulants, frequently referred to as sensitization, is currently being evaluated as a potential model for drug addiction and for drug-induced psychosis in humans (23,27). Numerous studies indicate that both the activating effects to many drugs of abuse, such as cocaine, amphetamine, and EtOH, as well as the expression of sensitization to those effects, involve direct or indirect action on the central dopaminergic neurons (9,20,24). Moreover, the locomotor activity has been the most frequently used measure to evaluate the stimulating effects and the behavioral sensitization of these drugs.

In the light of the above considerations, the present experiments were designed to assess the locomotor activating effects of the combination MZ+EtOH after acute and repeated injection in mice. We also examined the possibility that dopaminergic receptors are involved in the sensitization induced by these drugs by coadministering then with DA receptor antagonists.

METHOD

Animals

Male Swiss mice aged 90–100 days old and weighing approximately 35 g from our colony (UFSC) were used. The animals were housed in groups and maintained in a room with controlled temperature (23 ± 1°C) and a 12 L:12 D cycle. Food and tap water were available ad lib.

Drugs

The drugs used were mazindol (Medley, Brasil), ethanol proanalysis (Merck, Brasil), haloperidol and SCH-23390 (Research Biochemical, USA). Mazindol and haloperidol were dissolved in 0.025% carboximethylcellulose and diluted with distilled water. Ethanol was diluted in NaCl to a 12.5% v/v concentration. SCH-23390 was dissolved in distilled water. The control solution consisted of an equivalent volume of distilled water plus vehicle. All drugs were administered intraperitoneally (IP).

Apparatus and Procedure

Locomotor activity of each animal was measured in a cage of wood (40 × 12 × 20 cm) with steel grid floors and equipped with three parallel horizontal infrared beans positioned 2 cm above the floor and spaced evenly along the longitudinal axis. The activity cages were linked to a digital counter that recorded photocell bean interruptions. All experiments were conducted between 0800 and 1200 h.

Mice were divided into four groups receiving once-daily IP injections for 7 consecutive days: control solution, EtOH (1.2 g/kg), MZ (5.0 mg/kg), and EtOH+MZ. After a 2-day abstinence, the animals received a challenge dose with the same treatment. Injected mice were returned to their bedding-lined home or holding cages. On days 1, 7, and 10 of treatment, mice from all four groups were tested for activity, with data collected in four 5-min blocks, starting at different time intervals, 15, 30, 45, and 60 min after the injections. Following each 5-min blocks, mice were removed from the activity cages and housed in a holding cage. Mice were not adapted in activity cages prior to the treatment administration.

During the pretreatment phase, mice received two daily injections for 7 consecutive days. The first IP injection was either haloperidol (0.05 or 0.075 mg/kg), SCH-23390 (0.025 or 0.05 mg/kg), or control solution, followed 30 min later by a second IP injection, which was either EtOH (1.2 g/kg), MZ (5.0 mg/kg), EtOH+MZ, or control solution. Activity measures and procedures were the same as those described above for the preceding experiment. The doses of drugs used in the present study were established in our previous report (6), or were chosen from a dosage range reported in the literature.

Statistical Analysis

The influence of drug treatment (between-groups factor) and the days of the treatment (within-groups factor), as well as the interaction treatment × day, upon locomotor activity was evaluated statistically using a two-way analysis of variance (ANOVA) with repeated measures. Following significant ANOVAs, a Newman–Keuls test was used to compare each treatment with the corresponding control value. The probability value (p) less than 0.05 was considered statistically significant for all tests.

RESULTS

The effects of acute and repeated administration of control solution, EtOH, MZ, and the combination EtOH+MZ are illustrated in the Fig. 1. Preliminary data analysis by two-way ANOVA with repeated measures indicated a significant effect of drug treatment, $F(3, 40) = 32.43$, $p \leq 0.0001$, a significant effect of day of the test, $F(2, 80) = 12.92$, $p \leq 0.0001$, and a significant interaction between drug and day factors, $F(6, 80) = 5.56$, $p \leq 0.0001$. Subsequent Newman–Keuls tests indi-

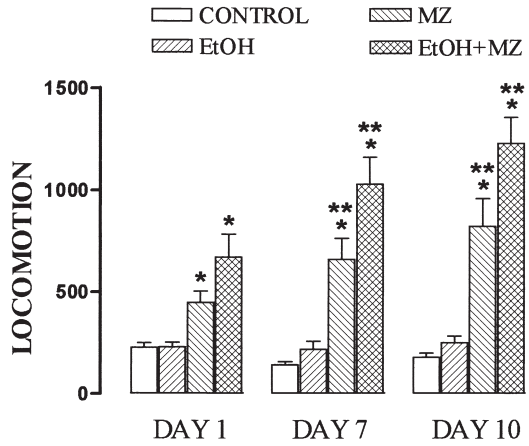

FIG. 1. Effects of control solution (control), EtOH (1.2 g/kg), MZ (5.0 mg/kg), or the combination EtOH+MZ, administered acute or repeatedly, on activity counts of mice. The locomotor activity was recorded for 5 min at different intervals following the treatments (15, 30, 45, and 60 min) at days 1 (acute), 7, and 10 (repeated administration). Values represent the mean ± SEM of 11 mice. *indicates $p \leq 0.05$ compared with control group and EtOH or MZ groups alone. **indicates $p \leq 0.05$ compared with the same treatment on day 1, Newman–Keuls test.

cated that MZ (5.0 mg/kg) alone significantly increased the locomotor activity over days, compared to control group. In addition, when compared to acute MZ locomotor stimulant effects (day 1), repeated administration of the drug induced a pronounced increase in this response (days 7 and 10). These results confirm the potential of MZ to induce behavioral sensitization in mice (Fig. 1). However, there were no significant differences between control animals and mice treated with the selected dose of EtOH (1.2 g/kg) throughout the activity testing days. The major finding of this study is that the locomotor activating effect of combined EtOH+MZ in mice was increased following repeated administration of drug combination, days 7 or 10 relative to day 1, suggesting sensitization ($p \leq 0.0005$, Newman–Keuls test) (Fig. 1).

The effects of haloperidol, a mixed DA receptors antagonist with higher affinity for D_2 receptors, on locomotor activity responses induced by EtOH and/or MZ are presented in Fig. 2. Two-way ANOVA with repeated measures indicated significant differences consequent to treatment factor, $F(11, 101) = 18.02$, $p \leq 0.0001$, day factor, $F(2, 202) = 7.18$, $p \leq 0.001$, and treatment × day interaction, $F(22, 202) = 5.13$, $p \leq 0.0001$. Further comparisons using Newman–Keuls tests confirmed that repeated exposure to MZ or to EtOH+MZ induces sensitization, which is characterized by an increased locomotor response to a subsequent drug challenge injection (Fig. 2). Only mice pretreated with a higher dose of haloperidol (0.075 mg/kg) before daily injections of the combination EtOH+MZ showed an attenuated stimulant responses across the 3 testing days. The same significant effect of haloperidol was detected in mice sensitized to locomotor effects by MZ alone. In addition, daily pretreatment with both doses of haloperidol slightly reduced the locomotor effects induced by EtOH alone, but these effects did not reach statistical significance (Fig. 2).

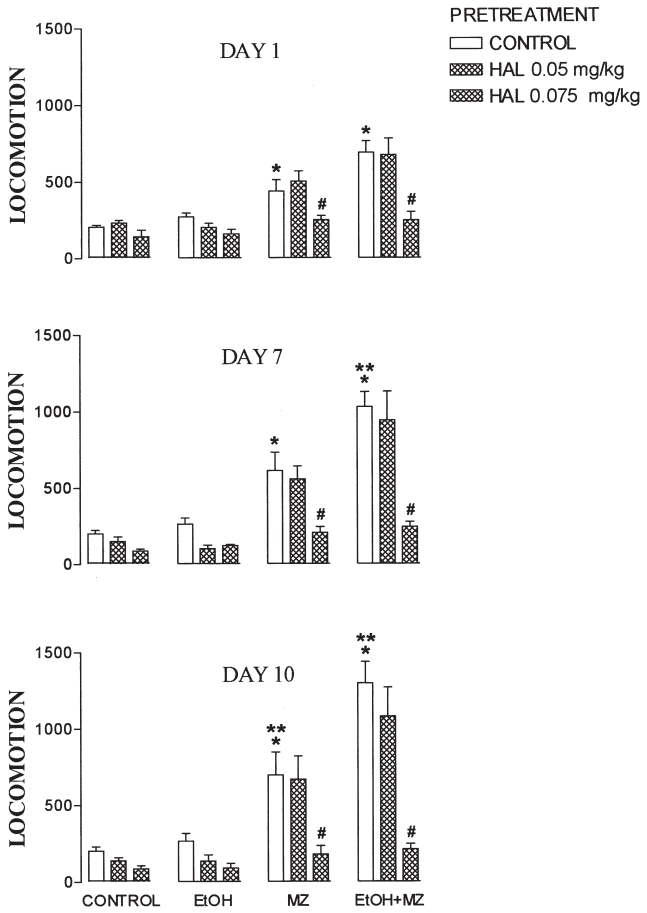

FIG. 2. Effects of haloperidol (0.05 and 0.075 mg/kg) on total activity counts induced by control solution (control), EtOH (1.2 g/kg), MZ (5.0 mg/kg), or the combination EtOH+MZ administered acute or repeatedly. The locomotor activity was recorded for 5 min at different intervals following the treatments (15, 30, 45, and 60 min) at days 1 (acute), 7, and 10 (repeated administration). Values represent the mean ± SEM of at least nine mice. *indicates $p \leq 0.05$ compared with control group and EtOH or MZ groups alone. **indicates $p \leq 0.05$ compared with the same treatment on day 1. #Indicates $p \leq 0.05$ compared with respective control treatment, Newman–Keuls test.

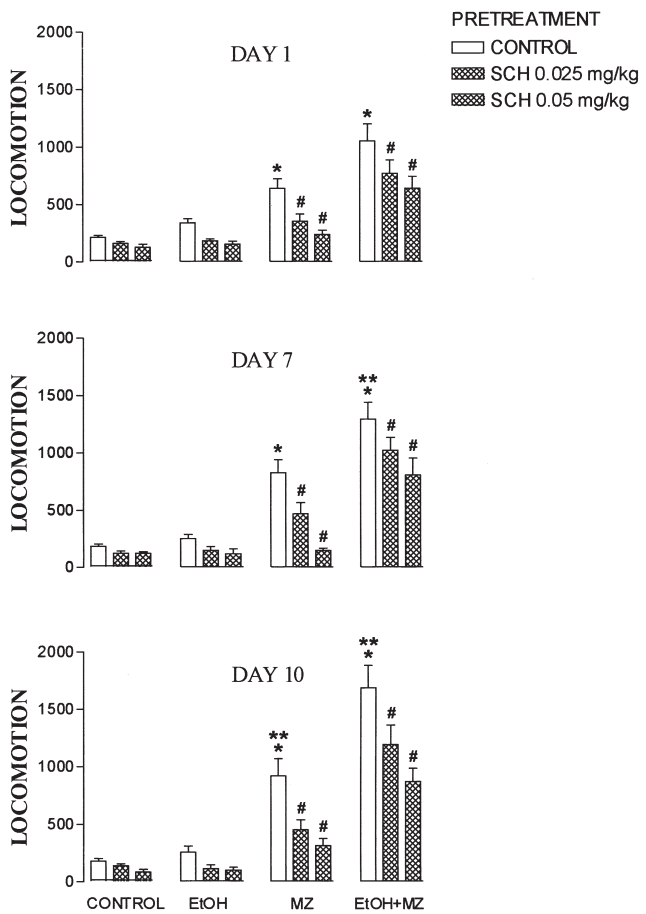

FIG. 3. Effects of SCH-23390 (0.025 and 0.05 mg/kg) on total activity counts induced by control solution (control), EtOH (1.2 g/kg), MZ (5.0 mg/kg), or the combination EtOH+MZ administered acute or repeatedly. The locomotor activity was recorded for 5 min at different intervals following the treatments (15, 30, 45, and 60 min) at days 1 (acute), 7, and 10 (repeated administration). Values represent the mean ± SEM of at least eight mice. *indicates $p \leq 0.05$ compared with control group and EtOH and/or MZ groups alone. **indicates $p \leq 0.05$ compared with the same treatment on day 1. #Indicates $p \leq 0.05$ compared with respective control treatment, Newman–Keuls test.

Figure 3 depicts the results of daily pretreatment with SCH-23390, a D_1 receptor antagonist, on the locomotor stimulating effects of MZ and EtOH+MZ. A two-way ANOVA with repeated measures demonstrated significant differences in treatment, $F(11, 99) = 23.31$, $p \leq 0.0001$, day factor, $F(2, 198) = 12.66$, $p \leq 0.0001$, and in the interaction treatment $\times$ day, $F(22, 198) = 6.02$, $p \leq 0.001$. Post hoc comparisons using Newman–Keuls test, also confirmed previous results indicating that mice repeatedly injected with MZ or EtOH+MZ showed a significant sensitivity to a challenge injection of the respective treatment. Most importantly, both doses of SCH-23390 significantly reduced the acute and the sensitized responses induced by the combined administration of EtOH+MZ (Fig. 3). Again, the same significant antagonism caused by SCH-23390 was observed towards the locomotor activating effects of MZ.

Finally, it is important to note that both dopaminergic antagonists, haloperidol and SCH-23390, at selected doses in this study, did not significantly alter the response of animals that received daily control solution injections (Figs. 2 and 3).

DISCUSSION

The present results confirm and extend our previous acute study showing that a interaction between EtOH and MZ may exist because the locomotor activity of mice injected with the combination was greater than the effects of either drug alone and that this response increased progressively over days, i.e., sensitization (6). The development of this sensitization to the combined drug administration is noteworthy because it may reflect the potential rewarding effects from the interaction of two different drug classes. Indeed, these findings suggest that a major determinant of combined anorectic + alcohol misuse may be the increased stimulating effects produced by such combination. In addition, the results indicate that coadministration of DA antagonists blocks the sensitization to both MZ alone and the combination EtOH+MZ.

The exact mechanism responsible for the interaction between EtOH and MZ following either acute or repeated administration remains unknown. It is likely that the results are mostly pharmacodynamic in nature. However, pharmacokinetic interaction cannot be ruled out, because MZ or EtOH plasmatic concentrations were not measured. Although sensitization to the locomotor activating effects of EtOH have been observed in different mouse strains (16,20,22), it is important to remember that this phenomenon is not as common for EtOH as it is for classical psychostimulants like cocaine and amphetamine. Besides, the selected dose of EtOH in the present study was low and nonstimulatory compared to the control group on day 1. Thus, the observed lack of sensitization following repeated administration of EtOH was not unexpected. Nonetheless, in recent years it has become increasingly apparent that a possible mechanism for some behavioral effects of EtOH involves stimulation of dopamine system (2,15,24). On the other hand, the anorectic MZ is known to act primarily, although not exclusively, by inhibiting dopamine uptake (8,21). In accordance to our previous research using rats, repeated daily injection of MZ induced a significant increase of locomotor activity over days, which was suppressed by DA antagonists. As mentioned earlier, most stimuli that result in sensitization either directly or indirectly appears to affect DAergic neurotransmission. Taken together, one might hypothesize that the prominent locomotor-stimulating effects by EtOH+MZ combination may stem, at least in part, from the enhanced DAergic activity in certain brain pathways produced by EtOH and MZ.

Consistent with this hypothesis, simultaneous treatment with either the selective D_1 antagonist, SCH-23390, or the mixed DA antagonist, with higher affinity for D_2 receptors, haloperidol, reduced the acute and repeated effects of EtOH+MZ on locomotor activity of mice. These results are also in accordance with previous studies that demonstrate that D_1 and D_2 antagonists attenuate the development of sensitization induced by DA agonists such as methamphetamine, fencanfamine, cocaine, amphetamine, and apomorphine (1,10,12–14, 25,26). In addition, the present findings confirm the notion that the expression of various dopamine agonist-induced behavioral effects requires the concomitant stimulation of both dopamine D_1 and D_2 receptors (3,5,17).

In conclusion, the present results demonstrate that repeated administration of MZ or the combination EtOH+MZ leads to sensitization of locomotor activity in mice. Moreover, the observed sensitization responses are blocked by pretreatment with either D_1 or D_2 receptor antagonists, suggesting that these results may reflect the known synergism of D_1-type and D_2-type action in many of the functional effects of psychostimulants. Also, in view of the prevalence of the reported misuse of anorectic in Brazil, these laboratory findings may contribute to the understanding of potential addiction problems following repeated MZ or the combination alcohol+MZ intake.

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