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Inhibition by Antimanic Drugs of Hyperactivity Induced by Methamphetamine-Chlordiazepoxide Mixture in Mice

KEISHI OKADA, RYOZO OISHI AND KIYOMI SAEKI[1]

Department of Pharmacology, Okayama University Medical School, Okayama 700, Japan

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OKADA, K., R. OISHI AND K. SAEKI. *Inhibition by antimanic drugs of hyperactivity induced by methamphetamine-chlordiazepoxide mixture in mice*. PHARMACOL BIOCHEM BEHAV **35**(4) 897–901, 1990.—The effects of lithium chloride and other antimanic drugs on locomotor hyperactivity induced by a mixture of methamphetamine (MAMP) and chlordiazepoxide (CDZP) were examined in mice, using an Animex activity meter. CDZP (12.5 mg/kg) given SC in combination with MAMP (1 mg/kg) caused a marked increase in locomotor activity, as compared with that in mice treated with MAMP alone. However, when CDZP (12.5 mg/kg) was administered together with 0.5 or 2.0 mg/kg of MAMP, no significant enhancement was observed. Lithium (2 and 3 mEq/kg, IP) and carbamazepine (4 and 8 mg/kg, IP) inhibited the hyperactivity induced by the MAMP (1 mg/kg)-CDZP (12.5 mg/kg) mixture to the level of activity in animals treated with MAMP (1 mg/kg) alone. Lithium and carbamazepine alone at these doses caused no significant inhibition of locomotor activity in saline- or MAMP-treated mice. Haloperidol (0.1 mg/kg, IP) and chlorpromazine (0.5 mg/kg, IP) decreased the MAMP-CDZP mixture-induced hyperactivity without significantly inhibiting locomotor activity in the saline- or MAMP-treated group. However, haloperidol (0.2 mg/kg) and chlorpromazine (1 mg/kg) alone significantly inhibited locomotor activity in all of the saline-, MAMP- and MAMP-CDZP mixture-treated groups. These results indicate that antimanic drugs selectively inhibit the hyperactivity induced by the MAMP-CDZP mixture, but that neuroleptics are less selective in inhibiting the hyperactivity.

Mania Methamphetamine Chlordiazepoxide Locomotor activity Haloperidol Chlorpromazine Carbamazepine Lithium

WHEN locomotor activity in rodents is determined, in an environment unfamiliar to the test animals, using a Y-maze or a hole board apparatus, the mixture of *d*-amphetamine (DEX) and chlordiazepoxide (CDZP) induces a state of hyperactivity much more marked than that produced by these drugs given separately (7,16). This intense hyperactivity is not observed when the DEX-CDZP mixture is administered to animals which have been acclimated to the apparatus (19). When an activity cage which is somewhat similar to the home cage is used, inconsistent results are obtained: the DEX-CDZP mixture-induced hyperactivity and the inhibitory effect of lithium on this hyperactivity, reported by Vale *et al.* (21), contrasts with the failures of U'Prichard and Steinberg (20) and Davies *et al.* (6) to observe such phenomena. The DEX-CDZP mixture-induced hyperactivity, as determined with a Y-maze or a hole board apparatus, is regarded as representing a kind of exploratory behavior which is generally observed when the rodents are taken out from their home cage and placed in an unfamiliar space (10). The hyperactivity induced by the DEX-CDZP mixture is inhibited by acute treatment with lithium at doses of 2–4 mEq/kg. Because, at these doses, this compound has no significant effect on locomotor activity in animals treated with either DEX or CDZP alone, DEX-CDZP mixture-induced hyperactivity has been used as an animal model for testing the behavioral effect of lithium (5, 9, 14, 21). However, with this model, the effects of antimanic drugs other than lithium have not been examined yet.

In the present study, we investigated the interaction between methamphetamine (MAMP) and CDZP in terms of the change in locomotor activity of mice as determined with an Animex activity meter, and the effects on excessive exploratory behavior induced by this drug combination of lithium and other drugs, such as carbamazepine and neuroleptics, whose antimanic efficacy has been shown clinically (2, 12, 13, 17), were examined in comparison with those on locomotor activity of mice given MAMP or CDZP separately.

METHOD

Animals

Male ddY mice weighing 25–35 g obtained from Seiwa

[1]Requests for reprints should be addressed to Dr. K. Saeki, Department of Pharmacology, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700, Japan.

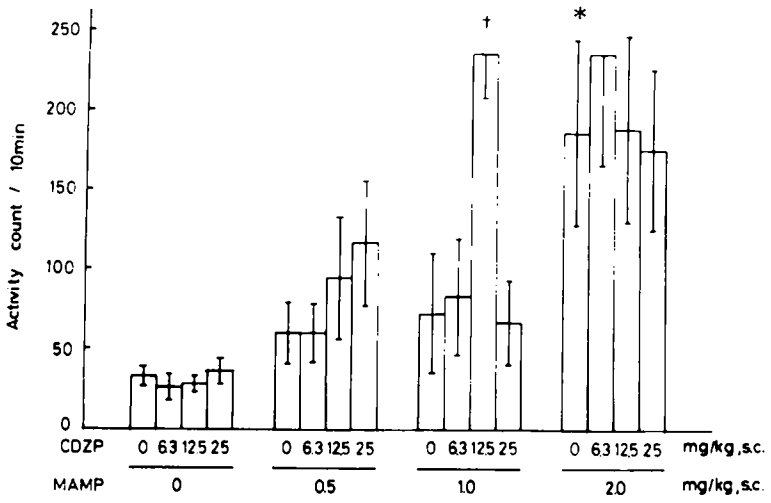

FIG. 1. Locomotor activity of mice given a combination of MAMP and CDZP. Mice were injected SC with the mixture of CDZP (0, 6.3, 12.5 or 25 mg/kg) and MAMP (0, 0.5, 1 or 2 mg/kg) and the locomotor activity was determined for the 10-min period starting 20 min after injection. Each column represents the mean ± SEM of 7 mice. *$p<0.05$ as compared with CDZP (0 mg/kg) plus MAMP (0 mg/kg)-treated group. †$p<0.05$ as compared with the corresponding CDZP (0 mg/kg)-treated group.

Experimental Animals (Fukuoka, Japan) were used. They were housed in groups at least 2 weeks after arrival in a room controlled at 22 ± 2°C and maintained in an alternating 12-hr light/dark cycle (lights on at 6:00). The mice were given free access to food and water. All experiments were carried out between 10:00 and 16:00.

Drugs

The drugs used in the present study were MAMP hydrochloride (Dainippon Pharmaceutical Co., Osaka, Japan), lithium chloride (Nakarai Chemicals, Kyoto, Japan), haloperidol (SERENASE injection, Dainippon Pharmaceutical Co.) and chlorpromazine hydrochloride (CONTOMIN injection, Yoshitomi Pharmaceutical Industries, Tokyo, Japan). CDZP and carbamazepine were donated by Takeda Chemical Industries (Osaka) and Ciba-Geigy Japan (Takarazuka, Japan), respectively. CDZP, carbamazepine and MAMP-CDZP mixture were suspended in 0.5% carboxymethyl cellulose (CMC) (Wako Pure Chemical Industries, Osaka) and other drugs were dissolved or diluted in 0.9% saline. They were injected in a volume of 10 ml/kg body weight. The doses of the salt-form drugs are expressed as the weight of the salts.

Locomotor Activity Measurement

Locomotor activity was determined by placing mice individually in a plastic cage (27 × 17 × 13 cm) put on an MK-Animex activity meter (model SE, Muromachi Kikai Co., Tokyo, Japan) and the activity counts were automatically put into memory in a PC9801 microcomputer (NEC, Tokyo) through an interface (Muromachi Kikai).

Statistical Analysis

The data were analyzed by the Mann-Whitney U-test. p Values of less than 0.05 were considered significant.

RESULTS

Effect of MAMP-CDZP Mixture on Locomotor Activity

Mice were administered SC with a combination of MAMP (0, 0.5, 1 or 2 mg/kg) and CDZP (0, 6.3, 12.5 or 25 mg/kg), and returned to their home cages. After 20 min they were placed individually in the plastic test cage on the apparatus and locomotor activity was counted for 10 min.

MAMP administered alone increased the locomotor activity in a dose-dependent manner and the effect was significant at 2 mg/kg (Fig. 1). On the other hand, CDZP given alone had no significant effect at any dose examined. In mice treated with the combination of 1 mg/kg MAMP and 12.5 mg/kg CDZP the hyperactivity was far more marked than that observed in mice treated with MAMP (1 mg/kg) alone and the difference was significant. However, no significant differences were observed between the groups injected with MAMP (0.5 or 2 mg/kg) alone and the groups injected with the mixture of MAMP (0.5 or 2 mg/kg) and CDZP (6.3, 12.5 or 25 mg/kg). Therefore, the mixture of 1 mg/kg MAMP and 12.5 mg/kg CDZP was used in the following experiments.

Time Course of the Effects of MAMP-CDZP Mixture and MAMP Alone on Locomotor Activity

The mice taken out of their home cages were injected SC with MAMP (1 mg/kg) alone or MAMP-CDZP mixture (1 and 12.5 mg/kg, respectively) and placed immediately in the plastic test cage on the apparatus. The locomotor activity counts were recorded for 5 min, every 10 min, until the fourth measurement, and are shown in Fig. 2.

During the first 5-min period starting immediately after the injection of MAMP or MAMP-CDZP mixture, no significant difference was observed between the two groups in locomotor activity counts. The level of locomotor activity in mice treated with MAMP alone did not change markedly until the fourth

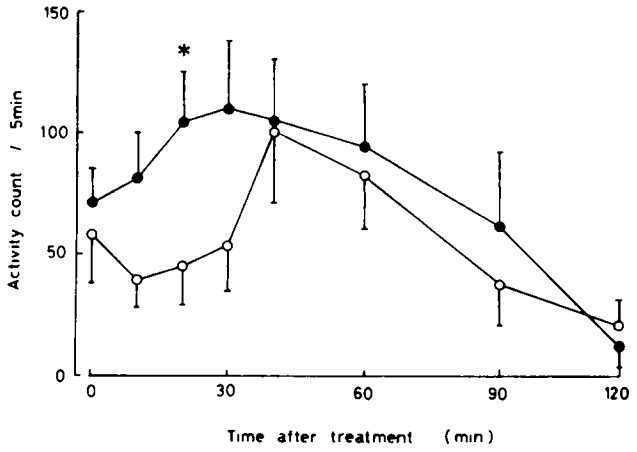

FIG. 2. Effects of MAMP alone (1 mg/kg, SC; ○) and MAMP-CDZP mixture (1 and 12.5 mg/kg, respectively, SC; ●) on locomotor activity. Each point represents the locomotor activity determined for each 5-min period starting at the time shown on abscissa. Each value is the mean ± SEM of 7 mice. *$p<0.05$ as compared with the corresponding value in the group treated with MAMP alone.

measurement (30–35 min after injection), but it then increased sharply to the peak value obtained at the fifth measurement (40–45 min after injection). On the other hand, in mice treated with the MAMP-CDZP mixture locomotor activity increased until the fourth measurement. During the period from 40 min to 120 min after injection, the locomotor activity gradually decreased in a similar way in the both groups. Based on these results, the locomotor activity during the first 30-min period after treatment was used in the following experiments.

Effect of Lithium Chloride on Locomotor Hyperactivity Induced by MAMP-CDZP Mixture

Mice were pretreated IP with lithium chloride (1, 2 or 3 mEq/kg) or saline and returned to their home cages. After 3 hr, they were injected SC with saline, MAMP (1 mg/kg) or MAMP-CDZP mixture (1 and 12.5 mg/kg, respectively) and the locomotor activity was measured for 30 min.

MAMP alone increased the locomotor activity, but the MAMP-CDZP mixture increased activity much more markedly than MAMP alone (Fig. 3). Lithium chloride had no significant influence on the locomotor activity in either the saline- or MAMP-treated groups. However, the locomotor hyperactivity induced by the MAMP-CDZP mixture was significantly reduced by 2 and 3 mEq/kg of lithium chloride to the level observed in mice treated with MAMP alone. Lithium chloride did not affect the locomotor activity of mice treated with CDZP alone (data not shown).

Effects of Haloperidol, Chlorpromazine and Carbamazepine on Locomotor Hyperactivity Induced by MAMP-CDZP Mixture

Mice were pretreated IP with the vehicle (saline or CMC), haloperidol (0.1 or 0.2 mg/kg), chlorpromazine (0.5 or 1 mg/kg) or carbamazepine (4 or 8 mg/kg) 20 min before an SC injection of saline, MAMP or MAMP-CDZP mixture. Locomotor activity for the succeeding 30-min period was determined.

Haloperidol, at a dose of 0.1 mg/kg, had no significant effect on the locomotor activity of mice treated with saline or MAMP alone (Fig. 4). However, this dose of haloperidol markedly inhibited the MAMP-CDZP mixture-induced hyperactivity. At a dose of 0.2 mg/kg, haloperidol significantly inhibited the locomotor activity of animals regardless of whether they were treated with saline, MAMP alone or MAMP-CDZP mixture.

Chlorpromazine, at a dose of 0.5 mg/kg, did not affect the

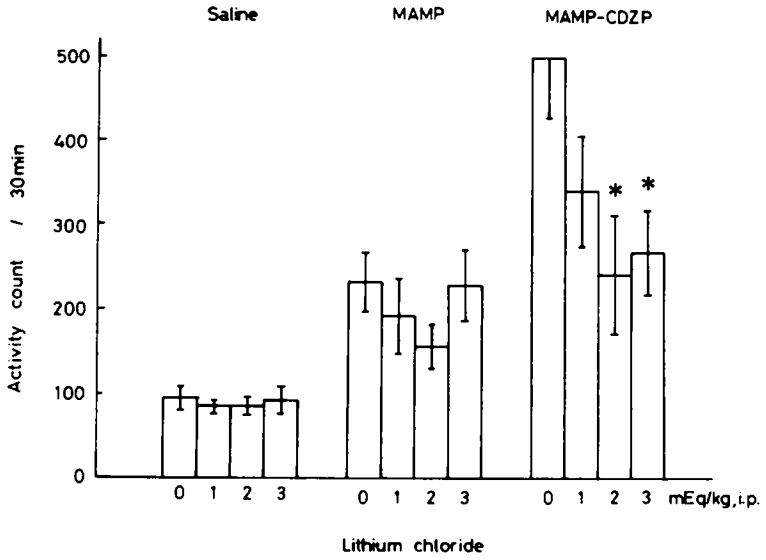

FIG. 3. The effect of lithium on locomotor activity of mice treated with saline, MAMP alone and MAMP-CDZP mixture. Mice were pretreated with lithium (0, 1, 2 or 3 mEq/kg, IP) 3 hr before SC injection of saline, MAMP alone or MAMP-CDZP mixture. The locomotor activity was determined for the 30-min period starting immediately after SC injection of drugs. Each column represents the mean ± SEM of 16 mice. *$p<0.05$ as compared with the corresponding control (lithium 0 mg/kg) group.

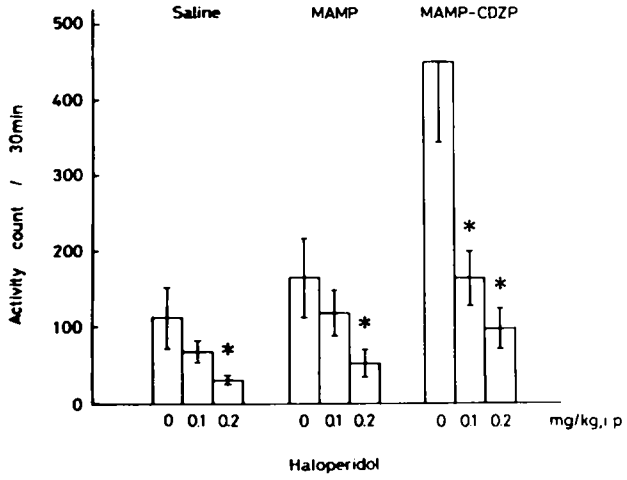

FIG. 4. Effect of haloperidol on locomotor activity of mice treated with saline, MAMP alone and MAMP-CDZP mixture. Mice were pretreated with haloperidol (0, 0.1 or 0.2 mg/kg, IP) 20 min before SC injection of saline, MAMP alone or MAMP-CDZP mixture, and the locomotor activity was determined for the 30-min period starting immediately after SC injection of drugs. Each column represents the mean ± SEM of 8 mice. *$p<0.05$ as compared with the corresponding control (haloperidol 0 mg/kg) group.

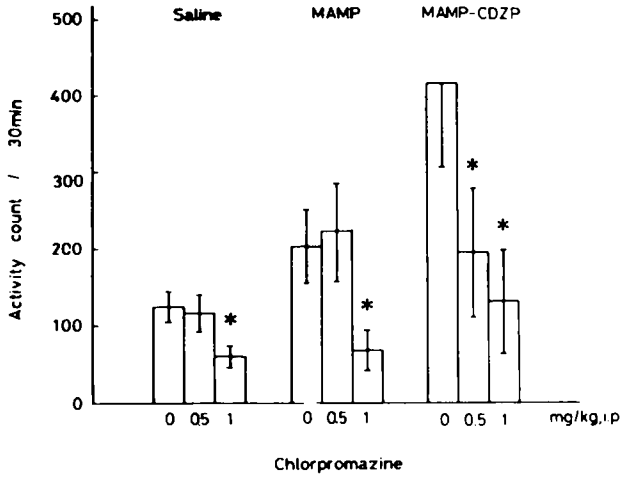

FIG. 5. Effect of chlorpromazine on locomotor activity of mice treated with saline, MAMP alone and MAMP-CDZP mixture. Mice were pretreated with chlorpromazine (0, 0.5 or 1 mg/kg, IP) 20 min before SC injection of saline, MAMP alone or MAMP-CDZP mixture, and the locomotor activity was determined for the 30-min period starting immediately after SC injection of drugs. Each column represents the mean ± SEM of 16 mice. *$p<0.05$ as compared with the corresponding control (chlorpromazine 0 mg/kg) group.

locomotor activity of mice treated with saline or MAMP alone. However, the same dose of chlorpromazine significantly decreased the hyperactivity induced by the MAMP-CDZP mixture to the level observed in the group given MAMP alone (Fig. 5). Chlorpromazine at 1 mg/kg significantly reduced the locomotor activity of animals regardless of whether they were treated with saline, MAMP alone or MAMP-CDZP mixture.

Carbamazepine at 4 and 8 mg/kg did not affect locomotor activity in the groups given saline or MAMP alone (Fig. 6). However, at these doses, this compound significantly decreased the MAMP-CDZP mixture-induced hyperactivity to the level of activity exhibited by animals given MAMP alone.

DISCUSSION

In the present experiment, the administration of a MAMP-CDZP mixture to mice induced a state of hyperactivity much more marked than that induced by MAMP alone, and the pretreatment with lithium reduced the level of activity in mixture-injected mice to that in mice injected with MAMP alone. These results are in good agreement with those obtained by previous investigators (5,14), who reported that lithium inhibited the DEX-CDZP mixture-induced hyperactivity as determined in a Y-maze and a hole board apparatus. In the present study the optimum doses of MAMP and CDZP in the mixture were shown to be 1 and 12.5 mg/kg, respectively. This is also in good agreement with the doses of DEX and CDZP (1.18 and 12.5 mg/kg, respectively) adopted for the combined use in previous experiments (5, 7, 16). Like the DEX-CDZP mixture, the MAMP-CDZP mixture did not enhance the activity to a level higher than that observed in mice treated with MAMP alone, when the animals had been acclimated to the plastic test cage by placing them in the cage for the 30-min period immediately before injection of the drugs (data not shown). Therefore, the mixture-induced hyperactivity may be regarded as representing a kind of excessive "exploratory" behavior. This same view has already been expressed (5,11).

In the present experiments, at doses of 1–3 mEq/kg, lithium did saline-treated mice, but it had a significant inhibitory effect on the MAMP-CDZP mixture-induced hyperactivity at 2 and 3 mEq/kg. Consistent with the present results, lithium, at doses of 2–4 mEq/kg, has been shown not to affect ambulation in otherwise untreated mice (3,8) and rats (5). Furukawa *et al.* (8) reported that lithium (1.58 and 4.72 mEq/kg, SC) inhibited the hyperactivity induced by 5 mg/kg of MAMP in mice and Berggren *et al.* (3) showed a slight inhibitory effect of lithium (4.1 mEq/kg, PO) on the hyperactivity induced by 3 mg/kg of DEX in mice. However,

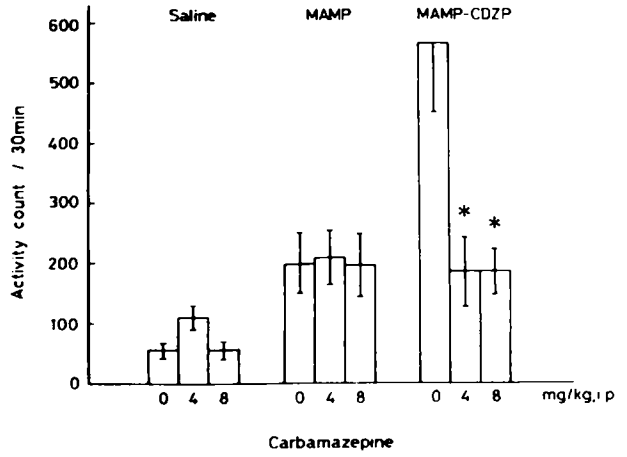

FIG. 6. Effect of carbamazepine on locomotor activity of mice treated with saline, MAMP alone and MAMP-CDZP mixture. Mice were pretreated with carbamazepine (0, 4 or 8 mg/kg, IP) 20 min before SC injection of saline, MAMP alone or MAMP-CDZP mixture, and the locomotor activity was determined for the 30-min period starting immediately after SC injection of drugs. Each column represents the mean ± SEM of 16 mice. *$p<0.05$ as compared with the corresponding control (carbamazepine 0

Cox *et al.* (5) observed no inhibitory effect of lithium (2 mEq/kg, IP) on locomotor activity of rats treated with 1.18 mg/kg of DEX. These discrepancies may be due to the differences in experimental conditions used, such as animal species, apparatus for the measurement of locomotor activity, doses of MAMP or DEX and so on. From these findings as a whole, it may be concluded that lithium decreases the MAMP-CDZP mixture-induced hyperactivity in a relatively specific manner. On the other hand, the inhibitory effects of neuroleptics, such as chlorpromazine and haloperidol, on the MAMP-CDZP mixture-induced hyperactivity seem to be less specific. These drugs decreased locomotor activity in mice treated with MAMP alone or saline at the same or slightly higher doses than those effective in reducing the MAMP-CDZP mixture-induced hyperactivity. Neuroleptics are clinically used in the treatment not only of schizophrenia, but also of manic states. Thus, neuroleptics are nonspecific in their therapeutic efficacy for these psychiatric disorders.

Clinically, carbamazepine has been suggested to have an antimanic action (2, 12, 13). In the present study, carbamazepine decreased the MAMP-CDZP mixture-induced hyperactivity, but did not influence the activity in mice treated with saline or MAMP alone. Smith (18) showed that acute treatment with carbamazepine even at a dose of 100 mg/kg (PO) did not influence the ambulatory activity of rats in an open field test. Therefore, carbamazepine seems to be more selective than lithium in inhibiting the mixture-induced hyperactivity. This may be of significance considering that carbamazepine appears to be more effective than lithium in some cases of severe mania, disphoric mania and rapid cycling illness (15).

The mechanisms by which the DEX-CDZP mixture induces hyperactivity are still unclear, although the involvement of catecholamines and serotonin in this excessive exploratory behavior has been suggested (1, 6, 9, 21). Poitou *et al.* (14) suggested a possible disturbance in norepinephrine-serotonin balance by the DEX-CDZP mixture and its prevention by lithium. Bunney and Garland-Bunney (4) proposed that a decrease in cholinergic activity, intervention in the dopaminergic system and inhibitions of cyclic AMP-mediated processes and phosphoinositol turnover may be related to the antimanic action of lithium. It remains to be determined whether lithium and carbamazepine inhibit the MAMP-CDZP mixture-induced hyperactivity via such mechanisms. The results of this study suggest that MAMP-CDZP mixture-induced hyperactivity may be of value as a model for screening antimanic drugs and also for elucidating the mechanisms of action of these drugs.

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