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# Effects of Methamphetamine and Ethanol on Learning and Brain Neurotransmitters in Rats

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YAMAMURA, T., S. HISHIDA, K. HATAKE, T. TANIGUCHI AND H. OUCHI. *Effects of methamphetamine and ethanol on learning and brain neurotransmitters in rats.* PHARMACOL BIOCHEM BEHAV 42(3) 389–400, 1992. – The interactions of methamphetamine (MAMP) and ethanol (EtOH) on multiple active/passive avoidance performance and neurotransmitters in different brain regions were examined. After the acquisition schedules, rats were retrained under the influence of MAMP (2 mg/kg/day, IP), EtOH (2 g/kg/day, IP), and in combination over 20 days in rats ($n$ = 6 per group). As a function of progress of drug treatment, MAMP–EtOH mixtures disrupt the learned avoidance performance and produced severe impairment of discriminative behavior caused by enhancement of excitability induced by MAMP when compared with MAMP only. At withdrawal, MAMP–EtOH-induced impairments of performance significantly persisted, whereas MAMP-only-induced impairments slightly recovered. At the eleventh day of drug withdrawal, MAMP-only-induced alterations of neurotransmitter levels at different regions were alleviated by EtOH, but these did not return to normal levels. These data provide support for the direct antagonistic and indirect additive interactions following constant daily treatment with a combination of MAMP and EtOH. EtOH may be an important factor in MAMP abuse to MAMP-induced psychosis or neurotoxicity.

Methamphetamine Ethanol Abuse Avoidance learning Neurotransmitters

SINCE cases of methamphetamine (MAMP) abuse have become increasing prevalent during the past 10 years in Japan, they have varied into multiple and vertical abuse in which the combined administration of different drugs to discover new psychoactive effects produces severe results and complex interactions. In such cases of abuse of sedatives, narcotics, tranquilizers, volatile solvents, etc., it is well known for the past 5 years or so that most drug abusers have a tendency to be willing to try any or all drugs, singly or in combination.

It is also indicated that the recent trend of increased frequency of ethanol (EtOH) use might introduce a phenomenon of two types of abuse patterns – one primarily concerned with the effects of MAMP and the other with the effects of EtOH. Most users try to make both of the drugs act as true antagonists (55), which tendency might suggest the two drugs were incompatible with each other.

It is commonly anticipated that MAMP or amphetamine (AMP), which has a similar pharmacological profile but is less toxic than MAMP and less nonselective (16,43), neutralizes the depressant effects of EtOH, while the excitability produced by MAMP (or AMP) is alleviated by EtOH. Since MAMP (or AMP) improves psychomotor performance and EtOH impairs it, one would postulate that the combination of them would produce performance intermediate between the two drugs (26).

The nature of EtOH addiction is such that EtOH develops reinforcing properties, that is, EtOH ingestion reduces anxiety or tension, and consequently it is easy to associate the psychotropic action of EtOH accordingly. Exposure to EtOH is a euphoria-inducing experience that lends itself to EtOH preference, which may produce a sort of pleasure, namely, that arising from the relief of discomfort caused by abstinence. On the other hand, MAMP (AMP) has the severe effect of creating a psychic dependence that induces a powerful primary reinforcer. These euphoric experiences induced by either MAMP (AMP) or EtOH alone would therefore alternate with each other in the subjective effects of both drugs. Conscious of the illegality of MAMP (AMP) use, abusers, in particular, may moderate their MAMP-induced excitability or they may be forced to compensate with EtOH as a substitute for MAMP. Thus, EtOH and MAMP (AMP) serve as cross-dependency drugs. Also, low-level continuous AMP adminis-

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tration selectively increases EtOH consumption (40), which suggests that the ordinary trend of chronic MAMP (AMP) abuse might be associated with coadministration with EtOH. As a result, the types of abuse patterns involving MAMP and EtOH are probably more widespread than have been previously supposed.

It is confirmed that chronic MAMP (AMP) administration causes a schizophrenic syndrome named MAMP (AMP) psychosis, including hallucination and illusion under clear consciousness (52), derived from a change of the brain monoamine activities (27,51,47). MAMP has been reported to cause a variety of changes in the central and peripheral neurotransmission systems (4,7,11,42). Repeated administration of MAMP (AMP) produces long-lasting changes in brain dopamine (DA), norepinephrine (NE) and serotonin (5-HT) systems. It was indicated that chronic MAMP (AMP) treatment was related to the inactivation of 5-HT systems, but more recent evidence of dopaminergic hyperactivity has been obtained after chronic MAMP administration, in addition to depletion of NE (12,44). On the other hand, chronic effects of EtOH manifested in alcoholics indicate that the chronic ingestion of EtOH may well have a profound effect on a large number of neurotransmitter-neuromodulator systems in the brain (22,36). Chronic administration of EtOH is associated with severe alterations in turnover of brain NE, release of DA, and content of 5-HT (18,53). It is hence strongly argued that the coadministration of both drugs would lead to greater complexities of interaction on brain neurotransmitter function that may in fact be more severe than has been previously supposed.

Few systematic studies have, however, investigated the effects of MAMP and/or EtOH on learned behavior in association with alterations of brain biogenic amines and their metabolites. It is likely that the combination of both drugs, in particular, induces a variety of behavioral responses with different alterations of brain neurotransmitters when compared to each drug treatment alone.

There are clearly a number of reasons for continuing to examine effects of mixtures of both drugs on learned behavior in rats because many MAMP abusers appear to administer both drugs (60) or show an inclination of EtOH preference (54,59). It is of interest to demonstrate whether the combined administration of MAMP and EtOH can be explained by direct depressant/stimulant antagonism on physiological alterations of brain neurotransmitters in relation to the performance of animals.

In the present study, operant conditioning behavior of rats was examined during daily treatment with MAMP or EtOH alone or in combination. the multiple active/passive avoidance schedule was used because it provides measures that indicate animals' behavioral characteristics on excitatory, inhibitory, and discriminative dimensions, involving three measurements (number of responses, shocks, and successes) (19, 20). Biological assays were conducted to measure alterations of neurotransmitters in different brain regions of rats after constant daily MAMP or EtOH alone or in combination.

## METHOD

### *Behavioral Assessment (Multiple Avoidance Training)*

*Animals and chemicals.* Twelve male Sprague-Dawley rats (160–190 g) were individually housed in plastic cages with free feeding for a minimum of 3 weeks before use in this study. Artificial lighting was on from 0800–2000 h, and temperature and humidity were kept at 24°C and 65%, respectively, throughout the experiments. Extraneous noise was diminished by enclosing the experimental room and operating a ventilation fan mounted on the outside of the room. Animals weighed 205–270 g at the beginning of each training experiment.

For intraperitoneal injection at a volume of 10 ml/kg body weight, MAMP sulfate (Dainihon Seiyaku, Tokyo) was dissolved in physiological saline solution and 20% (v/v) of EtOH solution was obtained from 99.5% (v/v) EtOH solution (Wako Junyaku, Osaka). Both drugs were mixed immediately before use for the mixed drug treatment. The doses of drugs stated in the text are those of the bases: 2 mg/kg for MAMP, 2 g/kg for EtOH, and combined drug treatment.

*Apparatus.* Two identical running wheels for avoidance training were used. Briefly, each wheel was made of clear Plexiglas, having a width of 12 cm and a diameter of 36 cm. The grid consisted of 0.5-cm stainless steel bars spaced 3 cm from center to center. A scrambled-shock current was transmitted to the grid of the wheel by means of a commutator in the hub of the wheel. Shock intensity was 200 V AC delivered through a 250-kΩ series resister. The wheel required a force of approximately 0.2 N applied tangentally to start it moving, and it could be turned in either direction. Rotation was detected by two capacitance switches. A quarter turn, or a circumferential running distance of 28.68 cm, defined a response. Repeated activation of the same switch by oscillation of the wheel was eliminated from the response counter by a flip-flop circuit. The wheel was housed in a wooden sound-attenuated box and illuminated during training sessions by a 100-V 10-W lamp that was attached to the inner ceiling of the box. Electromechanical equipment, located in the adjacent room, recorded responses and shocks.

*Procedure.* Rats underwent training for 10 sessions of multiple active/passive avoidance learning to confirm acquisition of avoidance behavior on alternate days. Rats were trained by means of a Sidman avoidance schedule. Shock duration was 0.5 s. In the active phase, animals had to keep running to avoid the occurrence of shocks, whereas in the passive phase they had to stop running. Each session was 80 min in duration, but the first 15 min of every session was a warm-up period during which no shock was presented. Both phases counterchanged every 300 s with presentation of a 100-V 10-W lamp light as a discriminative stimulus. The response-shock interval was 20 s, and the shock-shock interval was 5 s. When the responses (i.e., running) in the active phase and the degree of immobility in the passive phase were stable, the number of shocks was less than 10 in each phase, and the success rate (i.e., discrimination rate) was more than 80%, rats were regarded as drug-treatable subjects. They were randomly divided into four groups and the drug phase of the experiment was begun. On the first day, all rats were given intraperitoneal injections of the vehicle to accustom them to the injection procedure and establish baseline responses.

The maximum dose to be used for behavioral training was determined by means of the initial 6-day drug treatment, when animals received daily doses of MAMP that escalated as follows every 2 days: 2, 4, and 8 mg/kg. Because treatment with 4 and 8 mg/kg MAMP induced stereotypy and disrupted conditioned behavior, 2 mg/kg was determined as the optimum dose for the subsequent successive drug treatments (Fig. 1). EtOH was also administered IP in varying doses from 1–4 g/kg to groups of trained rats and their performance was monitored. The dose of 1 g/kg did not provoke a significant disruption of avoidance behavior. A prominent but very transient disruption of avoidance behavior was noted following the 2-g/kg dose. At 4 g/kg, the behavioral disruption was

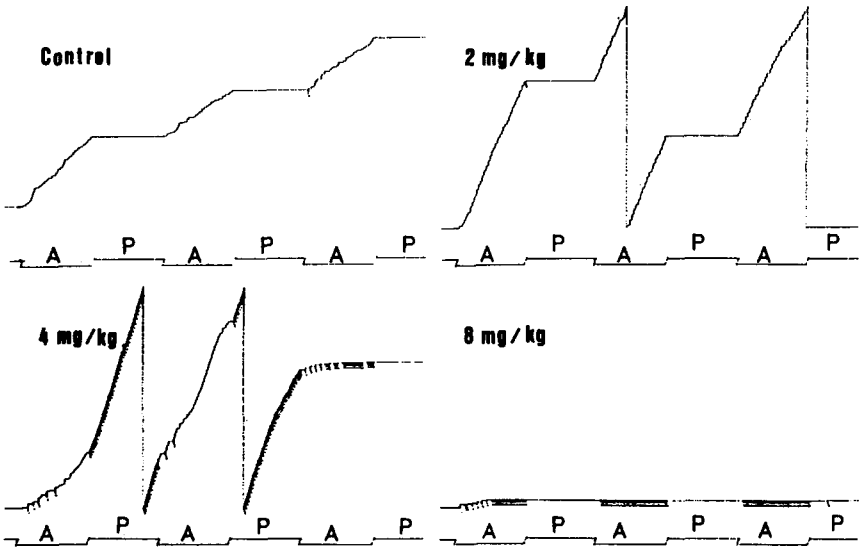


FIG. 1. Typical cumulative records in saline treatment session (control) and different methamphetamine treatment sessions (2, 4, and 8 mg/kg/day, IP) of multiple active/passive avoidance learning in rats.

greatly prolonged (Fig. 2). A dose of 2 mg/kg for MAMP and 2 g/kg for EtOH was chosen respectively as the dose to examine the effects of continuous single or combined drug treatment accordingly because none of the rats exhibited lasting effects of these initial drug treatments.

Beginning on the seventh day, the 2-mg/kg body weight MAMP dose, the 2-g/kg body weight EtOH dose, or the mixture were injected at 11:00 every day to each drug-treated group and an equivalent volume of physiological saline solution was administered to control rats. This procedure continued over 20 consecutive days with the multiple active/passive training taking place on alternate days during the drug treatment period. After the drug phase ended, training on alternate days continued for five sessions for determination of withdrawal effects of the drugs.

For each session of initial and drug-treated training, cumulative records of responses and shocks were collected by computer for analysis. The percentage of success was also calculated.

*Data analysis.* For the behavioral studies, differences between the mean values of each measure were analyzed using the Spearman's order coefficient and the two-tailed Mann-Whitney *U*-test. A difference with a probability of 0.05 was considered statistically significant.

### *Biochemical Assessment*

*Biochemical assay.* At the eleventh day after termination of constant daily drug treatment with MAMP (2 mg/kg, IP), EtOH (2 g/kg, IP), or the mixture (IP) for 20 days, rats were sacrificed by exposure to microwaves and whole brains, including the cortex (COR), hippocampus (HIP), striatum

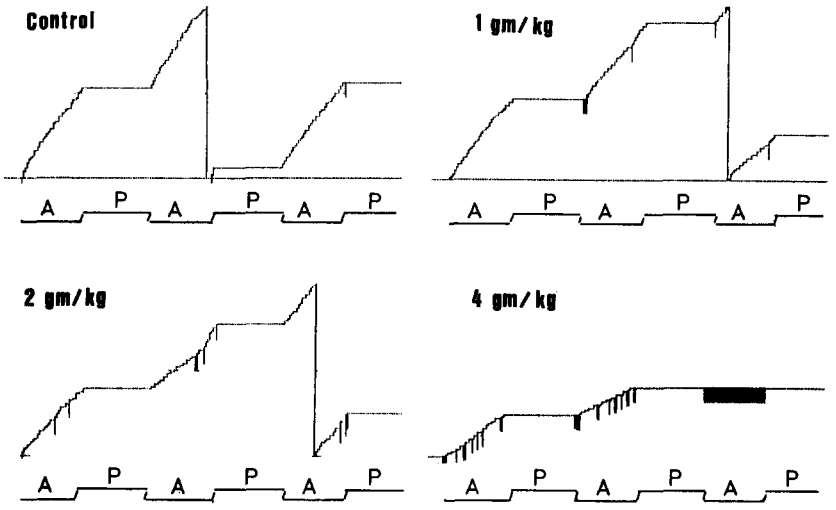


FIG. 2. Typical cumulative records in saline treatment session (control) and different ethanol treatment sessions (1, 2, and 4 g/kg/day, IP) of multiple active/passive avoidance learning in rats.

(STR) thalamus (THA), and hypothalamus (HYP) were immediately removed and rinsed with ice-cold saline. They were divided into each site, weighed, and homogenized with the adding of 500 μl/100 mg ice-cold 0.4 N perichloric acid solution. They were then centrifuged at $2 \times 10^4$ rpm for 15 min after staying in ice-cold condition for 30 min. Five microliters from each separated supernatant, diluted into twice volume by physiological saline, was injected into the high-performance liquid chromatography (HPLC) device. All procedures described above were conducted under ice-cold conditions.

Levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), NE, 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) were determined simultaneously by the HPLC procedure using an electrochemical detector Voltammetry: VMD-101, Yanagimoto Co. Ltd). The system consisted of an HPLC (model L-2000, Yanagimoto Co. Ltd) equipped with a reverse-phase column (Chemcopac, chemcosorb 7 ODS-H, 4.6 × 250 mm) used for separation of amines and a guard column. The mobile phase was 0.1 M sodium acetic acid and citric acid buffer at pH 3.9, containing 210 mg/l sodium octane sulfonic acid added to 4 mg/l EDTA, and the flow rate was set at 1 ml/min, yielding a pressure of 50 kg/cm$^2$. The concentration of monoamine in each sample was calculated using a standard curve of concentrations of monoamine and corrected for the recovery of the internal standard. The results are expressed in nanograms per gram of wet tissue.

*Data analysis.* For the biochemical studies, differences between the mean monoamine level were analyzed using the two-tailed Mann-Whitney *U*-test. A difference with a probability of less than 0.05 was considered statistically significant.

## RESULTS

### *Behavioral Results (Multiple Avoidance Training)*

Figures 3-5 show the responses, shocks, and success rates, respectively, during training and after drug treatment as a function of sessions. After the drug phase began, response stability was lost during the active period, and the immobility response during the passive period began to disappear, excluding treatment with EtOH alone. Responses under MAMP-only treatment were enhanced in the active period and especially in the passive period. Responses under mixture of MAMP and EtOH also increased in the passive period more than in the active period. Between MAMP-only and the mixed treatment, there were differences in time of occurrence of response deficits. Response deficits under the mixed administration were manifested at later sessions compared with MAMP alone. On the contrary, EtOH alone had no effect on responses. These tendencies of response deficits reflected an increase in shocks received. Conditioned discriminative responses were almost lost and stereotypic behaviors, in particular, appeared to be dominant.

In Fig. 3, the mean number of responses during active and passive periods as a function of drug and withdrawal sessions can be seen. In the active period, the mean number of responses did not vary but the stability of responding appeared confused during the initial sessions of MAMP-only or the mixed treatment. During the later sessions of MAMP-only or the mixed treatment, responses became enhanced, with a more rapid degree of increase induced by the mixed treatment than by MAMP only. In the passive period, both the mean number of responses and response instability were remarkably enhanced by MAMP-only and the mixed treatment. These disruptions in the passive period were eliminated rapidly in the withdrawal sessions, but in the active period response instability slightly remained after the end of MAMP-only and the mixed treatment. EtOH alone elicited a response decrease in the active period.

Figure 4 shows the mean number of shocks as a function of drug and withdrawal sessions. The mean number of shocks increased as a function of MAMP-only and the mixed treatment, especially during the passive period. In both active and passive periods, shocks declined smoothly during withdrawal.

Figure 5 shows the mean success rate as a function of drug and withdrawal sessions for the two periods separately. MAMP-only treatment reduced the success rate significantly for discrimination of active-to-passive periods (APP, $r_s = 0.533, n = 10, p < 0.05$) and passive-to-active periods (PAP, $r_s = 0.711$, $n = 10$, $p < 0.05$). EtOH-only treatment produced no significant effect on APP or PAP discrimination. On the other hand, the treatment MAMP + EtOH elicited a more severe reduction of the success rate for discrimination

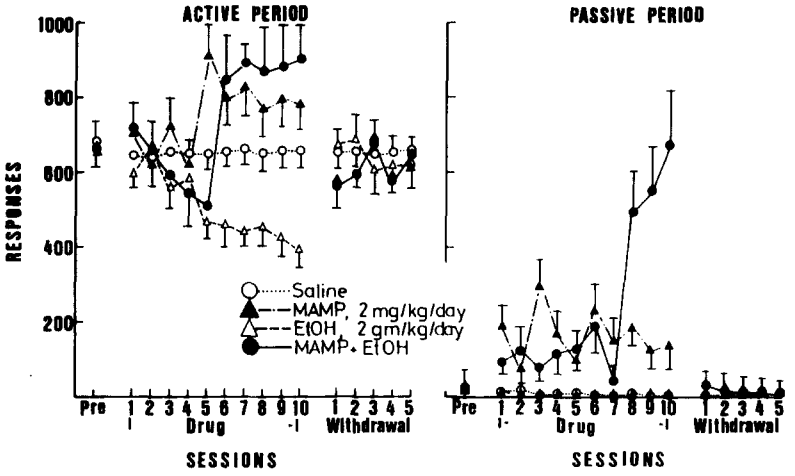


FIG. 3. Mean number of responses (±SEM) of rats treated with methamphetamine alone (MAMP: 2 mg/kg/day, IP), ethanol alone (EtOH: 2 g/kg/day, IP), in combination (MAMP+EtOH), or saline control over 20 days as a function of drug and withdrawal sessions during active and passive periods of multiple active/passive avoidance training on alternate days.

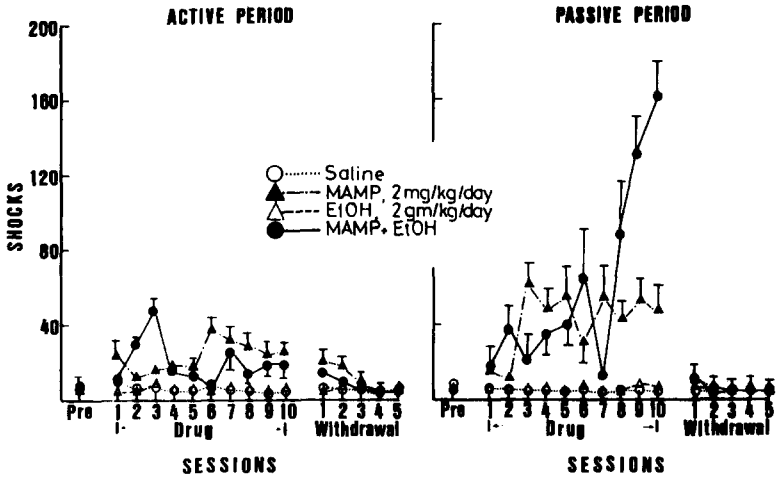


FIG. 4. Mean number of shocks (±SEM) of rats treated with methamphetamine alone (MAMP: 2 mg/kg/day, IP), ethanol alone (EtOH: 2 g/kg/day, IP), in combination (MAMP+EtOH), or saline control over 20 days as a function of drug and withdrawal sessions during active and passive periods of multiple active/passive avoidance training on alternate days.

of APP or PAP than MAMP-only treatment, although the success rate for PAP discrimination appeared to recover following progressive drug treatment. Success rate, however, showed a smooth recovery during withdrawal of EtOH treatment, excluding MAMP-EtOH and MAMP treatment, in which the persistence of reduced success rate was significant ($p < 0.05$), indicating a significant difference between APP and PAP periods ($p < 0.05$).

*Biochemical Results (Biological Assay)*

Figure 6 shows the effects of constant daily injection of MAMP, EtOH, the combination of both, and saline on concentrations of various neurotransmitters and their metabolites in rats' COR at 11 days after the last drug treatment. The DA concentrations in the COR were not significantly influenced by any drug treatment. An increase of NE concentrations in the COR to 181% was significant for constant MAMP-only treatment compared to the saline controls ($p < 0.01$). 5-HT concentrations with MAMP-only treatment decreased significantly to 18% compared to the controls ($p < 0.05$). MAMP-only treatment increased HVA levels to 172% ($p < 0.05$). 5-HIAA decreases to 64% were significant with MAMP-only treatment. In comparisons of drug treatment, a significant difference in concentrations of DOPAC in the COR was found between MAMP-only and EtOH-only treatment ($p < 0.01$).

Figure 7 shows the effects of each drug treatment and saline on various neurotransmitters in the HIP. DA and NE concentrations in the HIP increased to 223% and to 131%, respectively ($p < 0.05$), with MAMP-only treatment. 5-HT levels significantly decreased to 12% ($p < 0.01$) with MAMP-only treatment when compared to the saline controls. EtOH-only treatment produced a significant decrease of DA

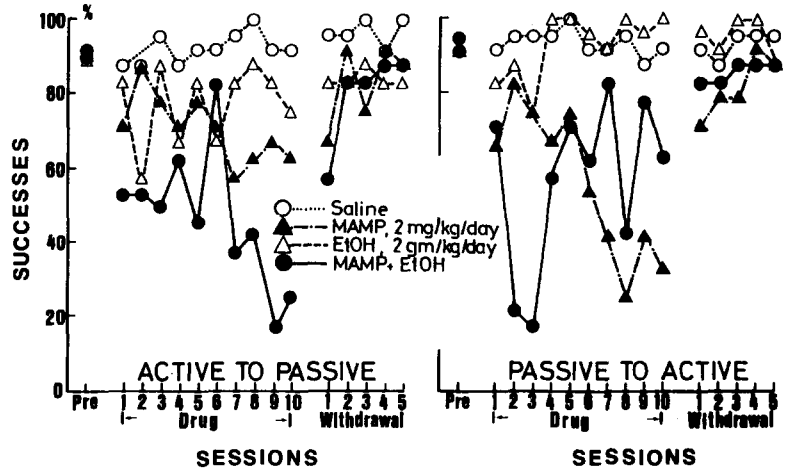


FIG. 5. Mean percentage of success of rats treated with methamphetamine alone (MAMP: 2 mg/kg/day, IP), ethanol alone (EtOH: 2 g/kg/day, IP), in combination (MAMP+EtOH), or saline control over 20 days as a function of drug and withdrawal sessions during active and passive periods of multiple active/passive avoidance training on alternate days.

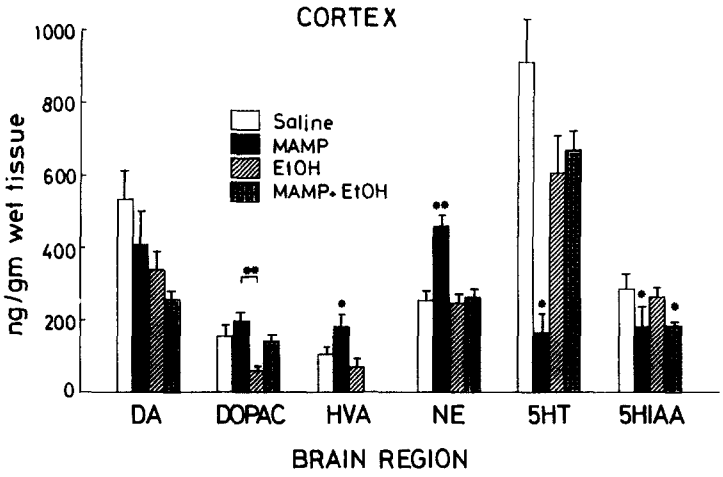


FIG. 6. Concentrations of various neurotransmitters and their metabolites in the rats' cortex (COR) at the 11 days following withdrawal from constant daily methamphetamine (MAMP: 2 mg/kg/day, IP), ethanol (EtOH: 2 g/kg/day, IP), the combination of both (MAMP+EtOH), and in saline controls treated over 20 days. The bars represent the mean (±SEM) for six rats. DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; NE, norepinephrine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid. $*p < 0.05$, $**p < 0.01$, compared to controls (two-tailed Mann-Whitney *U*-tests).

concentrations to 42% ($p < 0.01$), NE concentrations to 71% ($p < 0.05$), and 5-HT to 46% ($p < 0.01$) when compared to the controls. A decrease of 5-HT levels to 53% was significant in the MAMP + EtOH treatment group ($p < 0.05$). HVA concentrations showed a significant MAMP-only related increase of 335% ($p < 0.01$). A DOPAC increase of 263% was significant with MAMP + EtOH treatment ($p < 0.01$). When comparing drug treatments, a MAMP-only-induced decrease of 5-HT concentrations in the HIP was significantly different from a EtOH-only-induced decrease of 5-HT ($p < 0.05$). Comparing MAMP-only and MAMP-EtOH treatments, a significant difference in decreases of 5-HT levels was found ($p < 0.01$). Concentrations of DOPAC and 5-HIAA were significantly different between MAMP-only and EtOH-only treatments ($p < 0.01$).

Figure 8 shows the effects of drugs on various neurotransmitters in the STR. The DA concentrations showed a decrease to 44% with MAMP-only treatment ($p < 0.01$), to 71% with EtOH-only treatment ($p < 0.01$), and to 75% with MAMP + EtOH mixture treatment ($p < 0.01$) compared to control rats. In NE levels, an increase to 146% with MAMP-only treatment and a decrease to 58% with EtOH-only treatment

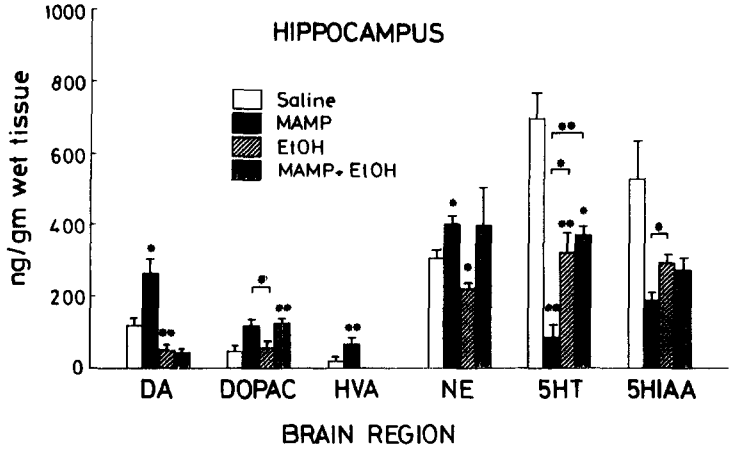


FIG. 7. Concentrations of various neurotransmitters and their metabolites in the rats' hippocampus (HIP) at the 11 days following withdrawal from constant daily methamphetamine (MAMP: 2 mg/kg/day, IP), ethanol (EtOH: 2 g/kg/day, IP), the combination of both (MAMP+EtOH), and in saline controls treated over 20 days. The bars represent the mean (±SEM) for six rats. DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; NE, norepinephrine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid. $*p < 0.05$, $**p < 0.01$, compared to controls (two-tailed Mann-Whitney *U*-tests).

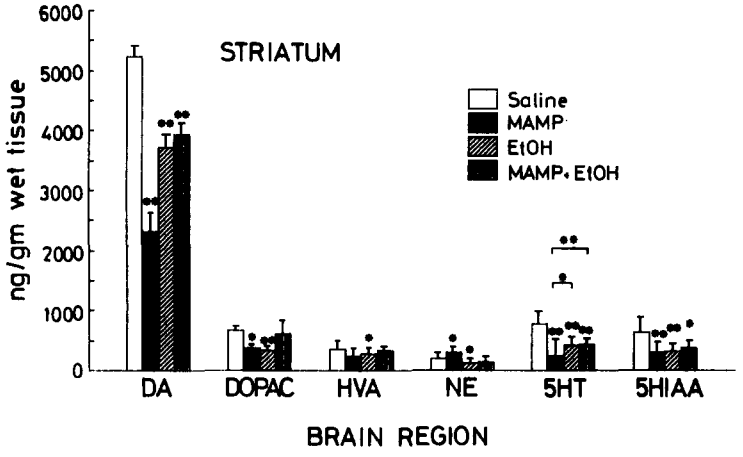


FIG. 8. Concentrations of various neurotransmitters and their metabolites in the rats' striatum (STR) at the 11 days following withdrawal from constant daily methamphetamine (MAMP: 2 mg/kg/day, IP), ethanol (EtOH: 2 g/kg/day, IP), the combination of both (MAMP + EtOH), and in saline controls treated over 20 days. The bars represent the mean (±SEM) for six rats. DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; NE, norepinephrine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid. $*p < 0.05$, $**p < 0.01$, compared to controls (two-tailed Mann-Whitney *U*-tests).

were significant ($p < 0.05$). The concentrations of 5-HT were reduced significantly to 31% by MAMP-only treatment ($p < 0.01$), to 54% by EtOH-only treatment ($p < 0.01$), and to 57% by MAMP + EtOH treatment ($p < 0.01$) compared to control rats. A significant decrease of 58% in DOPAC levels ($p < 0.05$) and of 48% in 5-HIAA ($p < 0.01$) was also indicated with MAMP-only treatment. EtOH-only treatment also significantly influenced DOPAC concentrations to decrease to 52% ($p < 0.01$), HVA to 79% ($p < 0.05$), and 5-HIAA to 52% ($p < 0.01$). 5-HIAA decreases to 60% were significant with MAMP + EtOH treatment ($p < 0.05$). Differences between MAMP-only and EtOH-only treatments on decreases of 5-HT levels in the STR were significant ($p < 0.05$). When comparing with MAMP-only and MAMP-EtOH treatments, a significant difference in concentrations of 5-HT levels was found ($p < 0.01$).

Figure 9 shows the concentrations of various neurotransmitters in the THA by each drug treatment. Changes in the concentrations of DA in the THA were not significant, but increases in DOPAC to 337% ($p < 0.05$) and HVA to 231%

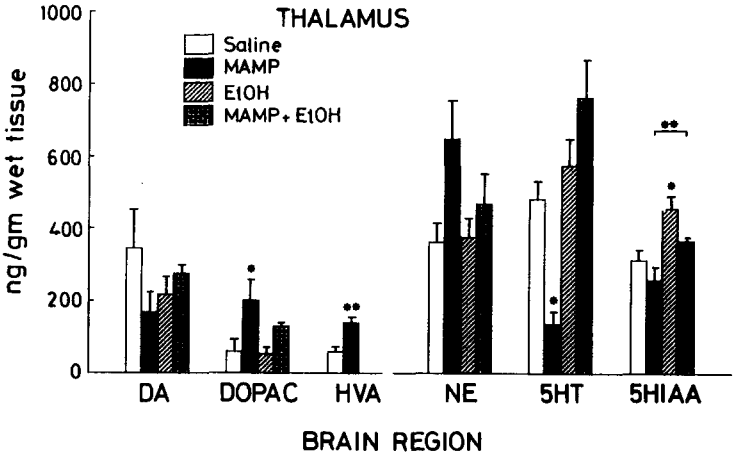


FIG. 9. Concentrations of various neurotransmitters and their metabolites in the rats' thalamus (THA) at the 11 days following withdrawal from constant daily methamphetamine (MAMP: 2 mg/kg/day, IP), ethanol (EtOH: 2 g/kg/day, IP), the combination of both (MAMP + EtOH), and in saline controls treated over 20 days. The bars represent the mean (±SEM) for six rats. DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; NE, norepinephrine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid. $*p < 0.05$, $**p < 0.01$, compared to controls (two-tailed Mann-Whitney *U*-tests).

($p < 0.01$) were significant with MAMP-only treatment. NE concentrations were not influenced by any drug treatment. 5-HT levels were significantly decreased to 28% in MAMP-only-treated rats ($p < 0.05$). 5-HIAA increases of 146% were significant with EtOH-only treatment ($p < 0.05$). Between MAMP-only and MAMP-EtOH treatments, 5-HIAA concentrations were significantly different ($p < 0.01$).

Figure 10 shows the effects of each drug on various neurotransmitters in the HYP. DA levels decreased significantly as a function of MAMP-only to 51% ($p < 0.05$). An increase in NE concentrations to 136% was significant with MAMP-only treatment ($p < 0.05$). A significant decrease in 5-HT concentrations to 39% was found with MAMP-only treatment ($p < 0.01$). EtOH-only treatment significantly decreased NE to 48% ($p < 0.01$) and 5-HT to 66% ($p < 0.05$). Both NE and 5-HT concentrations were significantly decreased to 63% ($p < 0.01$) and to 70% ($p < 0.05$) by MAMP-EtOH mixed treatment. DOPAC and HVA levels increased significantly as a function of MAMP only to 180% ($p < 0.05$) and 183% ($p < 0.01$), respectively. A decrease in 5-HIAA concentrations to 39% was significant ($p < 0.05$) with MAMP-only treatment. An HVA decrease to 54% was significant with EtOH-only treatment when compared with controls ($p < 0.05$). A MAMP-only-induced decrease of 5-HT levels was significantly different from an EtOH-only-induced decrease of 5-HT levels ($p < 0.01$). A significant difference in DOPAC concentrations was obtained comparing EtOH-only and MAMP-EtOH treatments ($p < 0.01$).

These results are summarized in Table 1, indicating that significant alterations of various neurotransmitters and their metabolites occurred as a function of treatment with different drugs. Concentrations of DA in two brain sites, excluding the HIP, were reduced by MAMP-only treatment, resulting from enhancement of DA release and its turnover and related to increases of HVA. Constant daily treatment with MAMP only resulted in increases of NE in almost all sites of brain. Reduction of both 5-HT and 5-HIAA levels in all brain sites was found after MAMP-only treatment. 5-HT turnover was remarkably augmented throughout the brain since 5-HIAA depletion was found with MAMP-only treatment. An effect on DA turnover was evident after constant daily EtOH-only treatment. EtOH-only induced decreases of DA concentrations in two sites of brain. Constant daily treatment with EtOH only resulted in decreases of NE concentrations in three sites of brain due to increased brain turnover of NE. Both 5-HT and 5-HIAA levels in most sites were decreased after EtOH-only withdrawal. MAMP-EtOH treatment induced a decrease of both DA and 5-HT concentrations in the STR. MAMP-EtOH treatment also decreased 5-HT levels in the HIP and the HYP. NE concentrations in the HYP were decreased by MAMP-EtOH treatment. Reduction of 5-HIAA levels in the COR and the STR was found with MAMP-EtOH treatment. MAMP-EtOH treatment increased DOPAC levels. A combination of MAMP and EtOH thus influences striatal DA and hypothalamic NE. It also induces severe depression of 5-HT levels in whole brain.

## DISCUSSION

A multiple active/passive training schedule in the running wheel situation induces avoidance performance that requires each rat to learn avoidance contingencies by which an active component of behavior is acquired as a conditioned response and a passive component can be acquired as an operant response (21). The present results dissociate effects of a mixture of MAMP and EtOH from either drug given separately on avoidance performance, including excitatory, inhibitory, and discriminative components of behavior. Some early studies on the effects of constant repeated treatment with a combination of stimulants like MAMP and depressants like EtOH predicted intermediate or antagonistic effects between the two drugs (6,10,15,46). When MAMP was given in combination with EtOH, however, no clear evidence of antagonism of EtOH was demonstrable on performance of human subjects stressed with a delayed audiofeedback system (17) or in a mental task performance situation (5). Enhancement of excit-

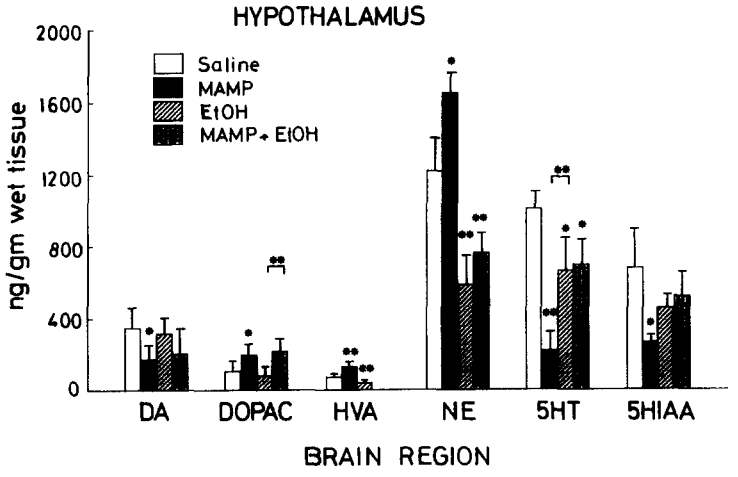


FIG. 10. Concentrations of various neurotransmitters and their metabolites in the rats' hypothalamus (HYP) at the 11 days following withdrawal from constant daily methamphetamine (MAMP: 2 mg/kg/day, IP), ethanol (EtOH: 2 g/kg/day, IP), the combination of both (MAMP + EtOH), and in saline controls treated over 20 days. The bars represent the mean (±SEM) for six rats. DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; NE, norepinephrine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid. $^{*}p < 0.05$, $^{**}p < 0.01$, compared to controls (two-tailed Mann-Whitney *U*-tests).

TABLE 1

EFFECTS OF CONSTANT DAILY TREATMENT WITH METHAMPHETAMINE (MAMP; 2 mg/kg/day, IP), ETHANOL (EtOH; 2 g/kg/day, IP), AND THE COMBINATION OVER 20 DAYS ON BRAIN REGIONAL NEUROTRANSMITTERS AND THEIR METABOLITIES IN RATS ($n$ = 6)

| | DA | | | | | DOPAC | | | | | HVA | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | COR | HIP | STR | THA | HYP | COR | HIP | STR | THA | HYP | COR | HIP | STR | THA | HYP |
| MAMP | – | ↑* | ↓† | – | ↓* | –§ | –‡ | ↓* | ↑* | ↑* | ↑* | ↑† | – | ↑† | ↑† |
| EtOH | – | ↓† | ↓† | – | – | –§ | –‡ | ↓† | – | –§ | – | × | ↓* | × | ↓* |
| MAMP+EtOH | – | – | ↓† | – | – | – | ↑† | – | – | –§ | × | × | – | × | × |

| | NE | | | | | 5HT | | | | | 5HIAA | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | COR | HIP | STR | THA | HYP | COR | HIP | STR | THA | HYP | COR | HIP | STR | THA | HYP |
| MAMP | ↑† | ↑* | ↑* | – | ↑* | ↓* | ↓†‡§ | ↓†‡§ | ↓* | ↓†§ | ↓* | –‡ | ↓† | –§ | ↓* |
| EtOH | – | ↓* | ↓* | – | ↓† | – | ↓†‡ | ↓†‡ | – | ↓*§ | – | –‡ | ↓† | ↑* | – |
| MAMP+EtOH | – | – | – | – | ↓† | – | ↓*§ | ↓†§ | – | ↓† | ↓* | – | ↓* | –§ | – |

All rats were sacrificed at 11 days after last drug treatment. COR, cortex; HIP, hippocampus; STR, striatum; THA, thalamus; HYP, hypothalamus; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; NE, norepinephrine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindole acetic acid.

↑, increase; ↓, decrease; –, no change; ×, not detected.

*$p < 0.05$, †$p < 0.01$, compared to controls (two-tailed Mann–Whitney $U$-tests).

‡$p < 0.05$, §$p < 0.01$, compared between each appendix (two-tailed Mann–Whitney $U$-tests).

ability was observed in a performance task paradigm in which dogs were treated with moderate doses of EtOH and AMP, compared with either drug alone (57). Other performance experiments for both animals (29,32,38) and humans (35,58) indicate more complex results. They were contradictory due to a tendency to lump experimental results for acute drug treatment with dose-related effects and to greater complexities of interaction across a greater range of measures. The results of the present study demonstrated additive effects of the two drugs. The combined effects of MAMP and EtOH appeared to be bipartile as a function of progressive mixed treatment. MAMP alone increased the number of responses at both active and passive periods. EtOH caused a decrease in the responses in the active periods. When these drugs were administered in combination, it was apparent that the facilitating effect of MAMP was counteracted by EtOH only during the initial drug treatment, but became even more enhanced than by MAMP alone in the later sessions. This facilitation was most conspicuous in the last session following the drug treatment. In the MAMP + EtOH group, the number of shocks in the passive period was similar to the number of responses, suggesting a temporary disruption of performance. This disruptive effect caused by MAMP–EtOH treatment was more evident for the rate of success as a discriminative component of behavior. In spite of differences existing between the active-to-passive and passive-to-active discrimination, MAMP alone decreased the success rate as a function of progressive drug treatment, but EtOH showed only a slight decrease in the success rate. The combination MAMP–EtOH induced a more severe disruption of success rate than did each drug alone. Furthermore, the retardation on success rate declined in the active-to-passive discrimination more than in the passive-to-active discrimination. The passive-to-active discrimination under MAMP–EtOH treatment appeared to recover over sessions, resulting from a predominant MAMP-induced excitability. Drug-induced impairments of the number of responses and shocks smoothly recovered after termination of drug treatment, while the reduction of success rate as a discriminative component of behavior persisted significantly at withdrawal. In the drug withdrawal period, the degree of recovery of MAMP-induced disruption seemed to be slightly inferior to that of MAMP–EtOH, although there was no significant difference between them.

The present behavioral results are in agreement with clinical conclusions that the additive effect of MAMP and EtOH is a reality, for example, instances of increased excitability in EtOH-inebriated patients who had concomitantly taken AMP (25,48). Although individual differences in EtOH sensitivity may vary to alleviate the AMP (MAMP)-induced excitability (50), the present results for constant daily MAMP–EtOH treatment cannot be explained by a straight stimulant/depressant antagonism; rather, they show an initial antagonism and a later synergism following constant daily drug treatment in an avoidance learning paradigm. These behavioral results may be related to the reports of metabolic studies that EtOH treatment markedly causes an increase in the concentrations of AMP in the brain (23) or internal organs (61) during acute drug treatment in rats. The storage of MAMP in the brain or other internal organs may reflect MAMP-induced excitability. In addition to this, recent reports suggest that degeneration occurs in brain neurons after chronic MAMP administration (3,49). The MAMP-induced endogeneous neurotoxin formation may be affected by adding EtOH since EtOH plays some important role in modulating MAMP metabolism.

Behavioral disruptions induced by a combination of both MAMP and EtOH could be explained by alterations of brain neurotransmitters. Most behavioral effects of MAMP and EtOH are mediated by DA, NE, and 5-HT transmitter systems in brain. Although there are various factors to consider in the interaction between MAMP and EtOH, for example, difference of dosage between two drugs, types and ways of MAMP abuse with EtOH consumption, neuronal damage caused by each drug, etc., there is no doubt that several neurotransmitter systems after chronic treatment with both drugs seem to be covary. The uptake and release of NE may interfere with dopaminergic mechanisms and the metabolism of DA may affect

serotonergic systems. Depletion of 5-HT may modulate NE activity. This speculation is strongly supported by present results through measurement of neurotransmitter levels in different regions of brain at withdrawal after constant daily intermediate application of MAMP and EtOH alone and in combination. For instance, the present data indicated that MAMP blocked reuptake and promoted release of the monoaminergic transmitters, while EtOH inhibited DA synthesis.

The present data indicated that in constant treatment MAMP was a potent indirectly acting amine. The MAMP-induced alterations of brain neurotransmitters occurred at different regions of the brain that were differentially affected by MAMP. The effects of MAMP alone on brain neurotransmitters were generally similar to those previously reported for MAMP (9,45,56). Consistent with previous findings (2,33,62), EtOH treatment also had several influences on concentrations of neurotransmitters at different brain regions. On the other hand, MAMP-EtOH treatment had less effect on different brain neurotransmitters in different sites of brain and seemed to be limited in effect to certain sites. Those findings might partially explain differential behavioral disruption due to constant daily MAMP-EtOH treatment compared to MAMP alone. They are, however, not sufficient to explain the potent disruption induced by a combination of both drugs in comparison with MAMP-alone treatment.

The present results demonstrate no clear evidence as to whether the interactions of MAMP as a stimulant and EtOH as a depressant are antagonistic or not. Comparing MAMP and EtOH, MAMP induces decreases in DA and 5-HT and an increase in NE at withdrawal. On the other hand, EtOH produces reduction of all neurotransmitters in brain. If both drugs are antagonistic, the MAMP-induced increase of NE should be alleviated by EtOH treatment. This was observed in alterations of NE levels in rats treated with MAMP-EtOH in the HIP and STR. NE levels in the HYP, however, did not recover from their EtOH-induced depression after treatment with a mixture of MAMP and EtOH despite the increase of NE by MAMP and the decrease of NE by EtOH. In addition, the interaction of both drugs was antagonistic on DA concentrations in the HIP but was not so in the STR and the HYP. The complexity of interactions between MAMP and EtOH in alterations of brain 5-HT concentrations was more contradictory when compared with each separate drug treatment. 5-HT levels in the HIP and STR indicated that the MAMP-EtOH mixture produced no antagonism between the two drugs because 5-HT was decreased by treatment with both drugs although the MAMP-induced decreased was less than the EtOH-induced decrease.

Drug-induced alterations of neurotransmitters in different areas of brain varied. Some areas were altered to the same extent as other areas while others were not. Alterations of brain neurotransmitters seem regionally specific, with larger alterations occurring in some areas than in others. The cause of regionally specific alteration of neurotransmitters produced by a drug is speculative, but may be related to the differential neurotransmitter systems or functions. The norepinephrinergic system has its own mechanism and is distinguished from the dopaminergic system, which is different from the serotonergic system. These systems fulfill their functions with their own metabolic pathways. Since their sensitivities to drugs are different, drug-induced alteration of neurotransmitters manifests complexity. At any rate, some of the present regionally differential interactions of MAMP and EtOH may support an additive rather than an antagonistic interaction of MAMP and EtOH. However, there is some room to admit an antagonism between MAMP and EtOH. Some of the present results may indicate that MAMP-induced effects on neurotransmitters at certain brain sites are alleviated by EtOH, indicating that the two drugs appear to be antagonistic.

In general, considering interactions between drugs the possible mechanisms through which these interactions may occur are obviously numerous and complex. Some possible mechanisms include direct interactions between the primary pharmacologic effects of the drugs involved and/or indirect interactions including effects on absorption, distribution, metabolism, and excretion, etc. Several interactions may take place simultaneously or sequentially between two drugs to confuse interpretations of drug interactions. Species and individual differences also influence them. Metabolites may cause different interactions than the parent substances. It is conceivable that the direct interactions between MAMP and EtOH may be antagonistic, whereas the indirect interactions may be additive. To clarify these speculations, well-controlled measurements over time are required because the withdrawal process on alteration of each neurotransmitter involves time dependency. The period of withdrawal may be related to the apparent antagonism between MAMP and EtOH since rebound or recovery of each neurotransmitter level may occur.

The present experiment showed that effects of constant daily treatment with a combination of MAMP and EtOH on brain neurotransmitters are different from the effects of MAMP alone. MAMP-induced alterations of neurotransmitters are sometimes alleviated by a combination of MAMP and EtOH. Present findings also support an additive interaction of MAMP and EtOH.

It is important to consider the administration schedule for investigations of neuronal dysfunctions induced by drugs. Rats treated in the present experiment were daily injected. Several reports indicate that alterations of neurotransmitters are more severe when intermittent drug treatment is used (28,39). Also, the recent finding that the administration of high doses of MAMP led to the in vivo production of 6-hydroxy-dopamine provided evidence for a mechanism of toxicity. 6-Hydroxydopamine is a dopaminergic neurotoxin that destroys cells through the generation of free radicals (31). MAMP exerted toxic effects on brain DA and/or 5-HT by mediation of free radical formation after constant AMP treatment. Increases of toxic effects of MAMP may occur with metabolic inhibition of hydroxylation of MAMP by EtOH treatment (8).

Several fatal intoxication cases due to AMP (MAMP) demonstrate that small amounts of AMP (MAMP) than had been previously supposed were found to be the cause of death at autopsy (1,34). It is postulated that the interaction of stimulant and depressant has some role in the induction of the fatal intoxication. Coadministration of MAMP (AMP) and EtOH may explain the fatal effects of MAMP (AMP) with relatively small doses that are not expected to induce lethal levels. MAMP (AMP)-induced deaths have been reported to accompany physiological or behavioral changes, for example, anorexia, hyperpyrexia, and hyperexcitability (14,37), which suggests that the MAMP (AMP)-induced effects on cardiovascular activities might be the cause of death (13,30,63). It has been indicated that the fatal effects were enhanced by acute treatment of both MAMP (AMP) and EtOH to rats (41,61) and in some postmortem cases (24). The strong association of EtOH intake with MAMP abuse might have an important role in the fatal effect of MAMP.

Future studies of MAMP-EtOH interactions must focus

on maintaining adequately controlled conditions for the data to show specific interaction on different behavioral components. Also, studies of chronic MAMP should standardize dose and duration of drug. Such studies must begin to shift emphasis toward brain regional or subregional characterization of functional parameters of transmitters (synthesis, turnover, release, etc.) and away from brain analysis of transmitter levels. Neurotransmitter balances may be more profoundly affected by MAMP-EtOH interactions than are aspects of the functions of a single transmitter. These should be evaluated carefully and the functional significance of disruptions in chemical mediator relationships explored by showing that pharmacological manipulations of brain transmitters that correct a deficit or restore a balance have some ameliorative action on the ravages of chronic EtOH use in MAMP abusers or vice versa.

In conclusion, a combination of MAMP and EtOH demonstrated no clear overall antagonism between the drugs, which suggests that coadministration of both drugs in MAMP abusers might produce more complex and severe clinical phenomena that MAMP alone.

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