Pharmacology Biochemistry and Behavior, Vol. 57, Nos. 1/2, pp. 167–172, 1997

0091-3057/97 $17.00 + .00

PII S0091-3057(96)00311-5

Behavioral Effects of N-Cyanomethylmethamphetamine, a Product Derived from Smoking Methamphetamine with Tobacco, in Mice and Rats

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Received 21 August 1995; Accepted 30 April 1996

SEKINE, H., S. NAGAO, H. KURIBARA AND Y. NAKAHARA. *Behavioral effects of N-cyanomethylmethamphetamine, a product derived from smoking methamphetamine with tobacco, in mice and rats.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 167–172, 1997.—The stimulant effects of N-cyanomethylmethamphetamine (CMMA), a product derived from smoking methamphetamine (MA) mixed in tobacco, were studied by observing stereotyped behavior and measuring spontaneous motor activity in mice and rats over 180 min CMMA, 1, 3 and 10 mg/kg IP, elicited strong stimulant-like effects which were almost equivalent to those produced by MA. Drug monitoring for 180 min in mouse and rat plasma revealed that the principal substances responsible for the stimulant effect of CMMA were MA and amphetamine (AP) which were metabolized from CMMA by the animal. There was a species difference in metabolism of CMMA between mice and rats. The major metabolites were MA and AP in mouse plasma, followed by N-formylmethamphetamine (FMA), whereas the major metabolite was FMA in rat plasma, followed by MA and AP. The differences in the stimulant effects of CMMA between mice and rats were discussed in relation to its metabolic fate in mice and rats. © 1997 Elsevier Science Inc.

N-Cyanomethylmethamphetamine Methamphetamine Amphetamine Stereotyped behavior Spontaneous motor activity Smoking drug abuse Drug metabolism

ALTHOUGH methamphetamine (MA) abuse has been a serious drug abuse problem in Japan since after World War II, in recent years it has also proliferated in western countries, especially in Hawaii (9) and in the West Coast of the United States (4). Generally MA abusers in Japan have taken it through intravenous injection. However, recently other abuse patterns, such as oral, snorting, rectal administration and smoking, have been increasing because of the AIDS epidemic (2).

Very little was known about the efficacy of MA inhalation and the pharmacological effects of pyrolysis products resulting from smoking MA mixed with tobacco. Our previous research (11,12,13) showed that when MA mixed with tobacco was smoked with a smoking machine, MA in tar was less than 20% of MA originally added, and a considerable number of pyrolysis products were produced. N-cyanomethylmethamphetamine (CMMA) (Fig. 1) (11), a new compound, was identified in tar as a major product obtained from the simulated smoking of tobacco containing MA. However, the pharmacological and toxicological effects of CMMA have remained unclear.

In the present study, stereotyped behavior (STB) and spon-

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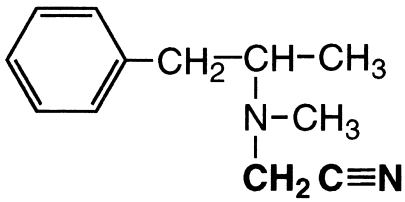

N-Cyanomethylmethamphetamine (CMMA)

FIG. 1. Chemical structure of N-cyanomethylmethamphetamine, a product of smoking methamphetamine with tobacco.

taneous motor activity (SMA) induced by CMMA in mice and rats was compared with that produced by MA. In addition, the relationship between the pharmacokinetics and the stimulant effect of CMMA was evaluated to estimate the principal role of CMMA versus its metabolites in producing stimulant-like behavioral profile. The differences in behavioral activity between mice and rats were also discussed in relation to the metabolic fate of CMMA in mice and rats.

METHOD

Subjects

Male ICR-SPF mice (Japan SLC Inc., Hamamatsu), 6–7 weeks old and weighing 28–31 g, and male Wistar SPF rats (Japan SLC Inc.), 6–7 weeks old, and weighing 190–205 g, were used. All animals were group housed (an animal per cage of 60 W × 30 D × 36 H cm) in an animal room with 12:12 h light/dark cycle (lights on at 0600–1800). The room temperature (22 ± 1°C) and relative humidity (55 ± 5%) were almost constant. Food (MF, Oriental Yeast Co., Tokyo) and tap water were provided ad lib. All experiments were performed with four to six animals in each group.

Drugs

CMMA hydrochloride and amphetamine (AP) sulfate were synthesized as previously reported (11). MA hydrochloride was purchased from Dainippon Pharmaceutical Co. (Osaka). Drugs were dissolved with saline, and injected IP at a volume of 0.1 ml/10 g and 0.2 ml/100 g body weight in mice and rats, respectively.

Behavioral Tests

Behavioral tests were performed between 0930–1300. The animals were used only once.

Stereotyped behavior (STB). Each of seven groups of mice and rats (n = 4–6) was given one of the following drug administrations: saline (control), 1, 3 and 10 mg/kg CMMA, or 1, 3 and 10 mg/kg MA, and the observation of STB was carried out at intervals of 10 min for 3 h. STBs were scored according to the rating scale proposed by Suzuki et al. (14); 0: Asleep, 1: Awake, usually not moving, occasional grooming and eating, 2: Short lasting locomotion and intermittent sniffing, 3: Continuous locomotion, rearing and sniffing, 4: Continuous sniffing and/or repetitive head and limb movement (brief periods of locomotion may be observed), 5: Intermittent licking, gnawing or biting of the cage, or self-biting, 6: Continuous licking, gnawing or biting the cage, or self-biting. In this experiment we defined scores of 3 or above as remarkable stereotyped behavior (STB ⩾ 3).

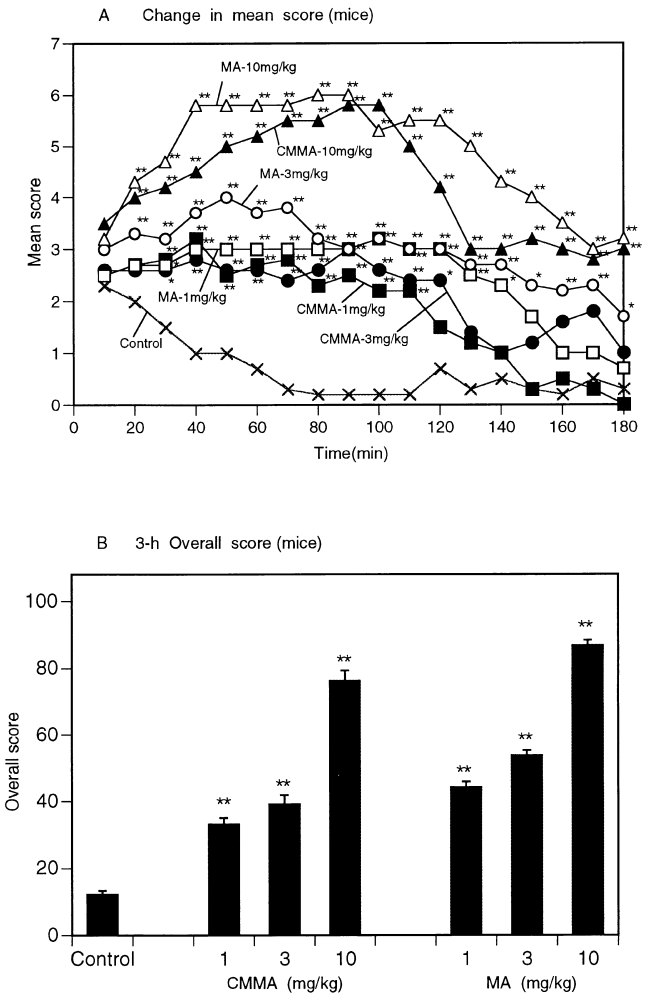

FIG. 2. Time courses of change in the mean scores (A) and the 3 h overall scores of stereotyped behavior with SEM (B) after IP administration of saline (control), N-cyanomethylmethamphetamine (CMMA: 1, 3 and 10 mg/kg), and methamphetamine (MA: 1, 3 and 10 mg/kg) to mice. n = 6 in each group except for groups given CMMA 3 mg/kg (n = 5) and 10 mg/kg (n = 4). * and **: $p < 0.05$ and $p < 0.01$, respectively, vs. saline-administered control (Scheffe test).

Spontaneous motor activity (SMA). Each of seven groups of mice and rats (n = 4 or 5) was given one of the following drug administrations: saline (control), 1, 3 and 10 mg/kg CMMA, and 1, 3 and 10 mg/kg MA. Immediately after drug administration, each animal was placed in a Plexiglas activity cage of 60 W × 30 D × 36 H cm, and its SMA was measured for 3 h with MK-Animex devices (Muromachi Kikai Co., Tokyo), with sensitivity of 40 μA for mice and 20 μA for rats, and tuning of 35–40 μA. The apparatus was set in a sound attenuating box.

Statistical Analysis

The data obtained from behavioral tests were analyzed by analysis of variance (ANOVA). The factors were the drug treatments (7 levels: 3 doses for CMMA and MA, and saline). In the cases where ANOVA yields a significant treatment

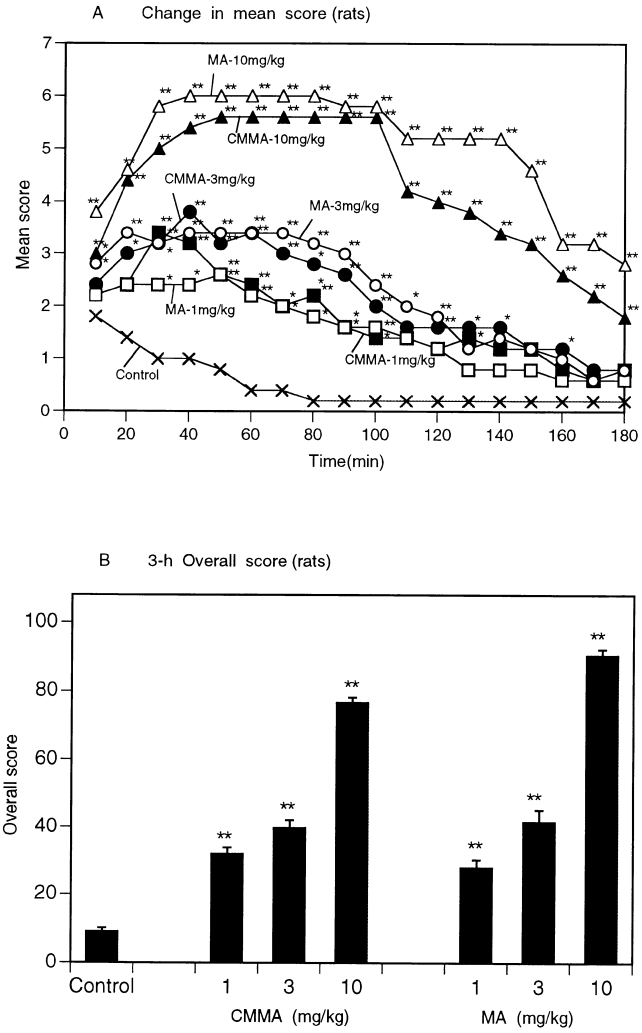

FIG. 3. Time courses of change in mean scores (A) and the 3 h overall scores of stereotyped behavior with SEM (B) after IP administration of saline (control), N-cyanomethylmethamphetamine (CMMA: 1, 3 and 10 mg/kg) , and methamphetamine (MA: 1, 3 and 10 mg/kg) to rats. $n = 5$ in each group. Data are presented in the same way as in Fig. 2.

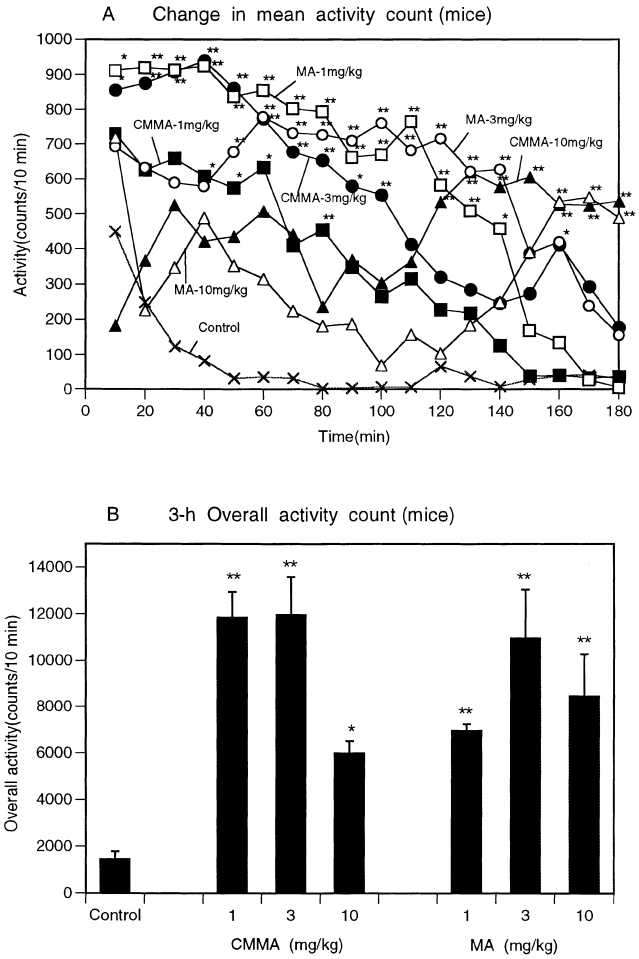

FIG. 4. Time course of changes in the mean SMA counts (A) and the 3 h overall counts with SEM (B) after IP administration of saline (control), N-cyanomethylmethamphetamine (CMMA: 1, 3 and 10 mg/kg), and methamphetamine (MA: 1, 3 and 10 mg/kg) to mice. $n = 5$ in each group. * and **: $p < 0.05$ and $p < 0.01$, respectively, vs. saline administered control (Scheffe test).

effect, post-hoc analyses were conducted by *t*-test with Bonferroni correction. Values of $p < 0.05$ were considered statistically significant.

Pharmacokinetic Study

Plasma sample preparation. At 5, 15, 30, 60, 120, and 180 min after the administration of 1, 3, 10 mg/kg CMMA, whole blood was collected by a guillotine in mice and from the orbital vein plexus in rats into an iced-cooled flask containing heparin. Plasma was obtained by centrifugation and stored at −20°C until analytical use. MA, 3 mg/kg, was also administered to rats and mice and the plasma samples were collected in the same manner described above.

Drug analysis. Ten μl of aqueous solution containing N-ethylamphetamine (EtA) at 20 μg/ml as an internal standard (IS) and 50 μl of 28% ammonium hydroxide (98:2) were added to the plasma (200 μl), and the mixture was extracted with n-propyl acetate (200 μl). The extracted solution was condensed under nitrogen stream to about 50 μl. One μl of the n-propyl acetate solution was automatically injected into the GC/MS. The selected ions were monitored at m/z 150, 119 and 91 for MA, 136, 119 and 91 for AP, 162, 97 and 189 for CMMA, 178 and 119 for FMA, and 164 and 119 for EtA (IS).

A Finnigan Mat Model Incos-50 gas chromatograph (GC)/mass spectrometer with a DB-17 column (J&W Scientific Inc., Folsom, California) 30-m long, with a 0.25 mm inner diameter (ID) and a 0.25-mm film thickness was used in chemical ionization mode with isobutane for determination of the metabolites. The oven was raised from 60°C to 240°C under a temperature program of 15 °C/min and held at 240°C for 2.5 min as mentioned in our previous paper (13).

RESULTS

Effects on STB

Mice. Figures 2A (upper panel) and 2B (lower panel) show time-courses of STB scores and overall STB scores for 3 h, respectively, after the administration of CMMA and MA to mice. STB scores were significantly dependent on time

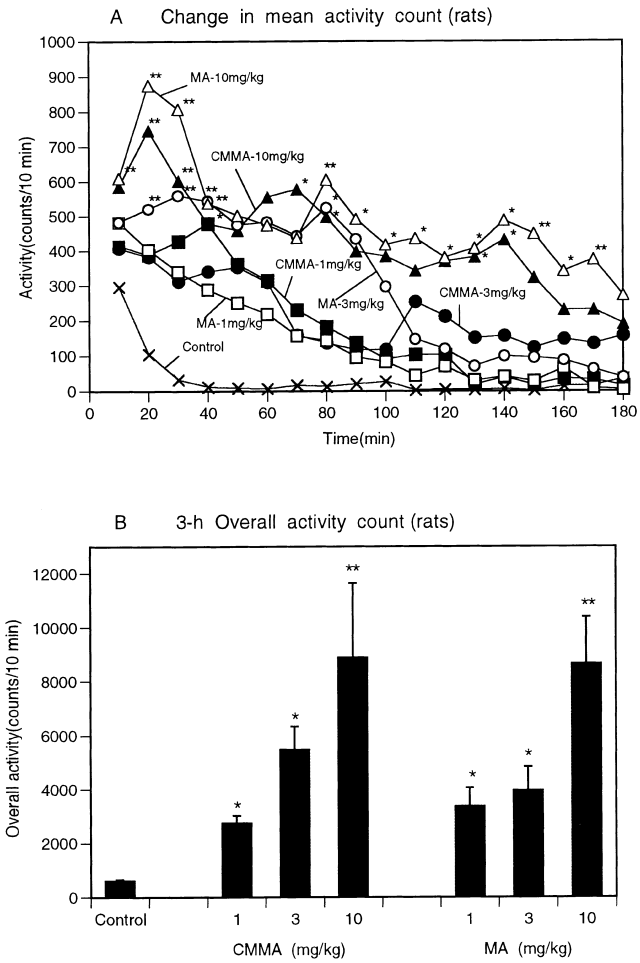

FIG. 5. Time course of change in the mean counts (A) and the 3 h overall counts with SEM (B) after IP administration of saline (control), N-cyanomethylmethamphetamine (CMMA: 1, 3 and 10 mg/kg), and methamphetamine (MA: 1, 3 and 10 mg/kg) to rats. $n = 4$ in each group. Data are presented in the same as in Fig. 2.

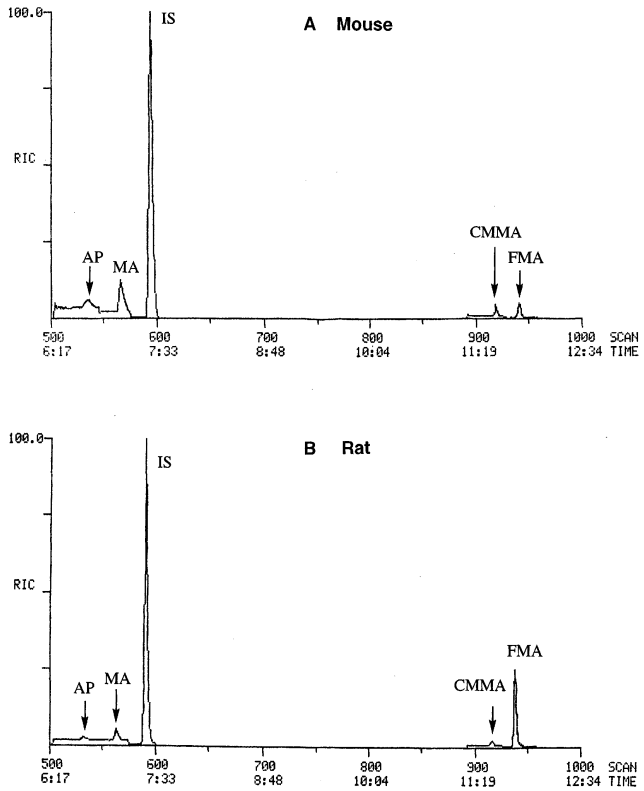

FIG. 6. GC/MS-SIM chromatograms of the extracts from mouse (A) and rat (B) plasma samples at 15 min after IP administration of N-cyanomethylmethamphetamine (CMMA: 3 mg/kg). AP: amphetamine, MA: methamphetamine, IS: N-ethylamphetamine, FMA: N-formylmethamphetamine.

[$F(17, 576) = 31.9$, $p < 0.001$] and dose [$F(6, 576) = 203.1$, $p < 0.001$], and there was a significant time × dose interaction [$F(102, 576) = 3.0$, $p < 0.001$]. The overall STB scores were also significantly dependent on dose [$F(6, 32) = 165.4$, $p < 0.001$]. Both CMMA- and MA-induced increases in STB scores were dose-dependent in both the maximum effect and the duration of effect. However, there were no significant differences in the scores of CMMA and MA throughout the 3 h observation period when the same dose of each compound was administered.

Rats. Figures 3A (upper panel) and 3B (lower panel) show time courses of STB scores and overall STB scores for 3 h, respectively, after the administrations of CMMA and MA to rats. The effects of time [$F(17, 504) = 41.4$, $p < 0.001$] and dose [$F(6, 504) = 402.7$, $p < 0.001$], and interaction between time × dose [$F(102, 504) = 2.4$, $p < 0.001$] were significant. The overall STB scores were also significantly dependent on dose [$F(6, 28) = 58.3$, $p < 0.001$]. Both CMMA and MA increased STB scores in a dose-dependent manner, and their potencies were almost the same.

Effects on SMA

Mice. Figures 4A (upper panel) and 4B (lower panel) show time-courses of SMA counts and 3 h overall SMA counts, respectively, after the administration of CMMA and MA to mice. SMA scores were significantly dependent on time [$F(17, 503) = 9.5$, $p < 0.001$] and dose [$F(6, 503) = 51.1$, $p < 0.001$], and there was a significant time × dose interaction [$F(102, 503) = 2.5$, $p < 0.001$]. The overall SMA counts were also significantly dependent on dose [$F(6, 28) = 8.1$, $p < 0.001$]. Both CMMA and MA increased SMA counts with the maximum effect at 3 mg/kg. SMAs caused by 10 mg/kg CMMA and MA were less than those induced by 3 mg/kg CMMA and MA, because of induction of strong stereotypy (see Fig. 2A). There were no significant differences in the CMMA- and MA-induced SMA.

Rats. Figures 5A (upper panel) and 5B (lower panel) show time-courses of SMA counts and overall SMA counts for 3 h, respectively, after the administration of CMMA and MA to rats. The effects of time [$F(17, 378) = 9.6$, $p < 0.001$] and dose [$F(6, 378) = 49.7$, $p < 0.001$] were significant. However, there was no significant interaction between time and dose [$F(102, 378) = 0.7$, ns]. The overall STB counts for 3 h were significantly dependent on dose [$F(6, 21) = 5.2$, $p < 0.01$]. Both CMMA and MA dose-dependently increased SMA counts with almost the same potency.

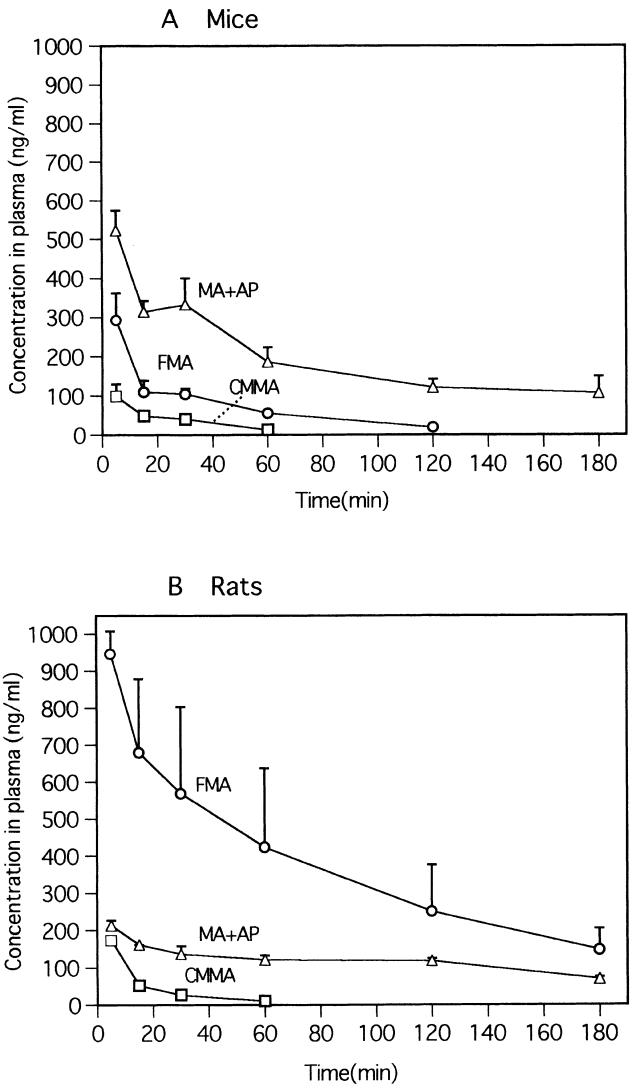

FIG. 7. Time course of changes in the mean concentrations ($n = 3$) of N-cyanomethylmethamphetamine (CMMA) , and its metabolites (amphetamine: AP, methamphetamine: MA, and N-formylmethamphetamine: FMA) in the mouse (A) and rat (B) plasma after IP administration of 3 mg/kg CMMA.

Active Metabolites of CMMA in Mouse and Rat Plasma

Figure 6 shows GC/mass chromatograms of the extracts from the mouse (Fig. 6A) and rat (Fig. 6B) plasma at 15 min after the administration of CMMA (3 mg/kg). In both the chromatograms, peaks of MA (7.1 min). CMMA (11.5 min), formyl-MA (FMA, 11.8 min) and AP (6.7 min) were observed, though MA was a major component in the mouse plasma, and FMA in the rat plasma.

Kinetics of Active Metabolites in Mouse and Rat Plasma After Administration of CMMA

Figures 7A and 7B show the time courses of change in the total active metabolites (sum of MA and AP) in mouse and rat plasma, respectively, over 180 min after the administration of 3 mg/kg CMMA. Evidently, the total levels of active metabolites in mouse plasma were higher than those in rat plasma. CMMA disappeared in the plasma of both species within 60 min after administration. The plasma level of an inactive metabolite, FMA, was much higher in rats than in mice.

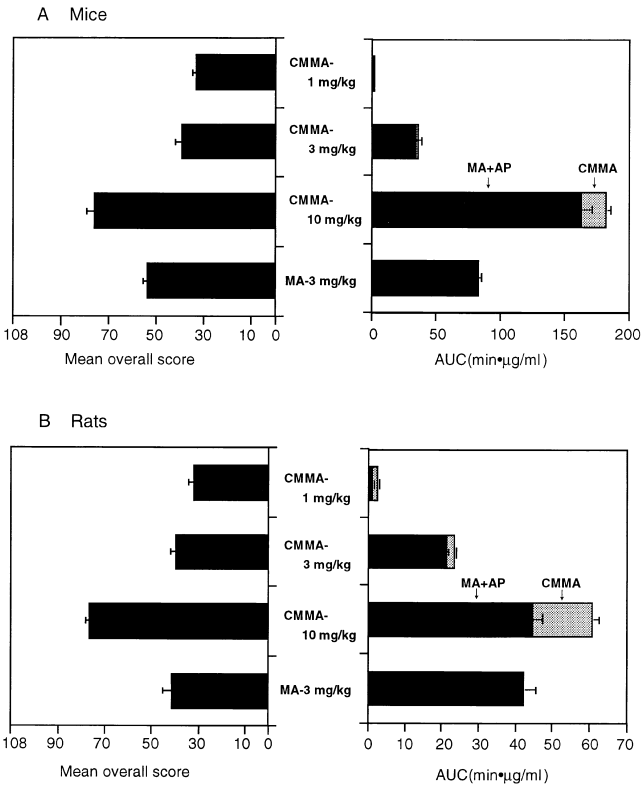

FIG. 8. Relationships between 180 min overall scores of stereotyped behaviors (STB) and AUCs of active metabolites (amphetamine plus methamphetamine) after IP administration of N-cyanomethylmethamphetamine (CMMA: 1, 3 and 10 mg/kg) and methamphetamine (MA: 3 mg/kg) to mice (A) and rats (B).

Relationship Between the Active Metabolite Levels in Plasma and STB Scores

As shown in Figs. 8A and 8B, there were close relationships between the total levels of the active metabolites (MA and AP, or MA and AP including CMMA) in plasma, and STB scores, suggesting these active metabolites played an important role in STB.

DISCUSSION

CMMA, the major product of smoking MA with tobacco (11,12), is an unique substance which has a cyanomethyl group at N position of MA. The present pharmacokinetic data revealed that CMMA was rapidly metabolized to MA in mice and to FMA in rats. It is unknown whether CMMA itself shows CNS actions similar to those observed by injection of MA. However, our experiments demonstrated that IP administration of CMMA to mice and rats induced STB and acceleration of SMA, clearly suggesting that CMMA has CNS stimulant actions which are qualitatively similar to those of MA. It has been reported (10,14) that at the dose range of 1–10 mg/kg, AP and MA lead a dose-dependent increase in STB, and following the administration of 10 mg/kg MA or AP to rats,

sniffing is accompanied by licking and biting. The increase of STB sometimes results in an interference of the expression of SMA (5,6). Thus, administration of the highest dose (10 mg/kg) of CMMA to mice and rats elicited a transient enhancement, followed by a decrease, of SMA. At a later point, there was a reappearance of a strong SMA. A strong STB including continuous licking, biting and gnawing (STB score 6) was observed during the decrease in SMA, which is consistent with previous reports (5,6) describing that the strong STB interfered with SMA. The comparison of the CMMA- and MA-induced STB revealed that CMMA has a stimulant effect with a similar potency and duration as that of MA.

It has not been clear whether CMMA could elicit stimulant-like effects by directly affecting CNS, or if its active metabolites, MA and AP, are responsible for the behavioral stimulation until the present study. The results of present pharmacokinetic study supported the latter consideration. Thus, following the administration of 3 mg/kg CMMA, the plasma CMMA levels decreased to nearly zero within 60 min, whereas levels of the total active metabolites (MA + AP) in the plasma of mice and rats were present for a long period. Along with the persistence of active metabolites in the plasma, a significant increase in the STB scores and SMA counts persisted for 120 min after the administration of 3 mg/kg, and for longer than 180 min after 10 mg/kg. Furthermore, there were dose dependent relationships between the overall scores of STB and AUCs of MA + AP or MA + AP including CMMA.

The major metabolite of CMMA was MA in mice and FMA, a putatively inactive metabolite, in rats. The present pharmacokinetic study demonstrated that after the administration of the same dose of CMMA, the plasma level of total active metabolites in mice was approximately 2 times as high as that in rats. Moreover, the relationship between STB scores and AUCs of total active metabolites showed that the mice were approximately 2 times less sensitive than the rats to the active metabolites. These results were consistent with the previous report concerning the differences in the sensitivities to MA between mice and rats in terms of ambulatory activity (8) and active avoidance response (7).

Although further studies are required to elucidate the pharmacological characteristics of CMMA, the following two conclusions can be derived from the present results. 1) The peripherally administered CMMA elicits behavioral excitation as strong as that produced by MA and AP. 2) The behavioral effects of CMMA are caused mainly by its active metabolites, MA and AP.

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