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The Effect of Optical Isomers of 3,4-Methylenedioxymethamphetamine (MDMA) on Stereotyped Behavior in Rats

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HIRAMATSU, M., T. NABESHIMA, T. KAMEYAMA, Y. MAEDA AND A. K. CHO. *The effect of optical isomers of 3,4-methylenedioxymethamphetamine (MDMA) on stereotyped behavior in rats.* PHARMACOL BIOCHEM BEHAV **33**(2) 343–347, 1989.—The relative potencies of S(+)-, R(−)-3,4-methylenedioxymethamphetamine (MDMA) and S(+)-methylenedioxyamphetamine (MDA) in inducing stereotyped behavior were determined in comparison with *p*-chloroamphetamine. S(+)-MDMA was more potent than R(−)-MDMA in eliciting stereotyped behaviors such as sniffing, head-weaving, backpedalling and turning and wet-dog shakes. These results are consistent with the actions of the drug on release of neurotransmitters in which the S(+) enantiomer is more potent. The desmethyl derivative of (+)MDMA, (+)MDA, was more potent than (+)MDMA in eliciting stereotyped behaviors, and produced wet-dog shake behavior.

Methylenedioxymethamphetamine (MDMA) Methylenedioxyamphetamine (MDA) *p*-Chloroamphetamine (PCA) Stereotyped behavior

(±)METHYLENEDIOXYMETHAMPHETAMINE (MDMA) is a 3,4-methylenedioxy-substituted phenylisopropylamine structurally related to CNS stimulants such as amphetamine, and to hallucinogens such as mescaline. Known to abusers as Ecstasy or Adam, MDMA has recently received considerable attention due to its widespread recreational use and subsequent emergency classification as a Schedule I drug by the U.S. Food and Drug Administration.

As might be expected from its chemical structure, MDMA elicits behavioral effects characteristic of both the amphetamines and the hallucinogenic phenylalkylamines (17). High doses of (±)MDMA were found to cause a dramatic depletion of serotonin (5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), as well as a decrease in the activity of tryptophan hydroxylase, the rate-limiting enzyme for 5-HT synthesis (15,18). The compound also causes selective neurodegeneration in a number of serotonergic terminal regions including the striatum, hippocampus and cerebral cortex in a manner similar to the 5-HT releaser, *p*-chloroamphetamine (2, 3, 13, 18).

Although the compound has been known for some time, the mechanism for its behavioral effects and its neurotoxicity is not well understood. Most of the experiments studying the behavioral effects of MDMA employed the discriminative stimulus effect paradigm common to psychoactive drugs (5,14). While this procedure is sensitive and specific in classifying drug action, it is not useful in determining the mechanism of the 5-HT neurotoxicity associated with high doses of MDMA. Since high doses of the serotonergic neurotoxin, *p*-chloroamphetamine, produce stereotyped behaviors that include head-weaving, backpedalling and turning (12,19), it seemed possible that high doses of MDMA might also cause similar behavioral alterations that would be useful as an alternative approach to the study of its interaction with neurotransmitter systems. Because of the continuous nature of behavioral responses, the time course of drug action could be monitored as well. Although MDMA is an optically active substance, little is known about the relative potency of its optical isomers in these behavioral effects. In other pharmacological evaluations, the two enantiomers exhibit different relative potencies. For example, S(+)-MDMA is approximately three times more potent than its R(−)-enantiomer in producing hyperthermia in rabbits (1). On the other hand, R(−)-MDMA is three to five times more potent than S(+)-MDMA in binding to central serotonin binding sites (9). Recently, Glennon *et al.* (5) have reported that S(+)-MDMA was four times more potent than R(−)-MDMA in a schedule-controlled response in mice. This manuscript describes results of studies comparing the stereotyped behavioral responses of enantiomers of MDMA and its desmethyl derivative, S(+)-MDA in comparison with *p*-chloroamphetamine.

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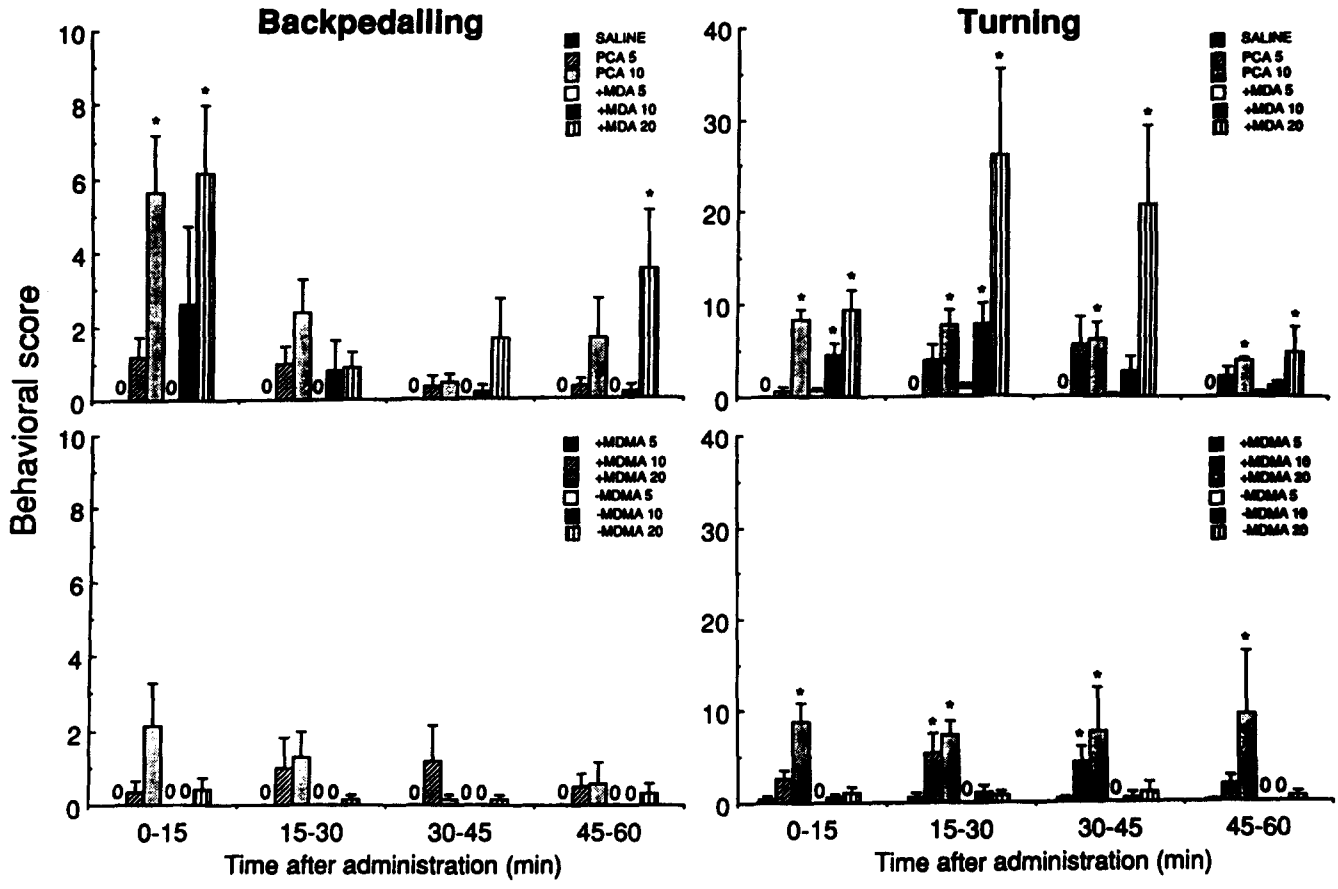

FIG. 1. Comparison of turning and backpedalling induced by *p*-chloroamphetamine (PCA), S(+)-MDMA, R(−)-MDMA and S(+)-MDA in rats. Rats were given PCA 5 or 10 mg/kg, SC or S(+)-MDMA, R(−)-MDMA and S(+)-MDA 5, 10 or 20 mg/kg, SC and stereotyped behaviors of each rat were recorded as described in the Method section. Values are the means ± S.E.M. of 7–9 animals. *$p<0.05$ vs. saline-treated group (Mann-Whitney U-test).

METHOD

Animals

Male Wistar rats (Kyoto Inst. Kitayama Lab. Co., Japan), weighing 200 to 300 g at the time of the stereotype experiments, were housed in a temperature (23 ± 1°C) and humidity (55 ± 5%) regulated room with a 12-hr light-dark cycle; the room was lit between 8 a.m.–8 p.m. for at least 5 days before the start of experiments. The animals were given free access to food and water.

Drugs

S(+)-MDMA, R(−)-MDMA and S(+)-MDA were obtained from the National Institute on Drug Abuse (Research Triangle, NC). *p*-Chloroamphetamine was obtained from Sigma, MO. Control experiments were performed by the subcutaneous injection of 0.9% saline solution. Doses of these drugs were expressed in terms of the HCl salt. Drugs were injected subcutaneously in a volume of 2 ml/kg body weight.

Measurement of Stereotyped Behavior

To evaluate stereotyped behavior, the behavioral scoring system developed by us (11) was employed and behavioral scores were recorded for four periods of 15 min each as follows: Sniffing (0: absent, 1: occasional, 2: frequent, 3: constant); head-weaving (the number of times the animal made slow, side-to-side or lateral head-movements); turning (the number of times the animal circled laterally to left or right over 360° within a relatively small area); backpedalling (the number of times the animal made backward locomotion). Stereotyped behaviors were observed in a cage with dimensions of 30 × 35 × 17 cm. During the observation periods, the total number of wet-dog shakes, including whole body shake and head shake, was counted. The experiments were conducted in a quiet laboratory. All results were expressed as the means ± S.E.M.

Statistics

All data were subjected to statistical analysis and the statistical significance was determined using Mann-Whitney U-test. The difference between two comparable sets of results was considered significant when $p<0.05$.

RESULTS AND DISCUSSION

MDMA and other mind-altering drugs have been compared by a number of different behavioral paradigms such as drug discrimination (5,14), hyperthermia (1), and stereotypy (present paper). These responses are produced at different doses and may be mediated by different neuronal systems. The behavioral response used here involves dopamine (DA) and 5-HT systems and allows a temporal assessment of activity which, in turn, can be ultimately

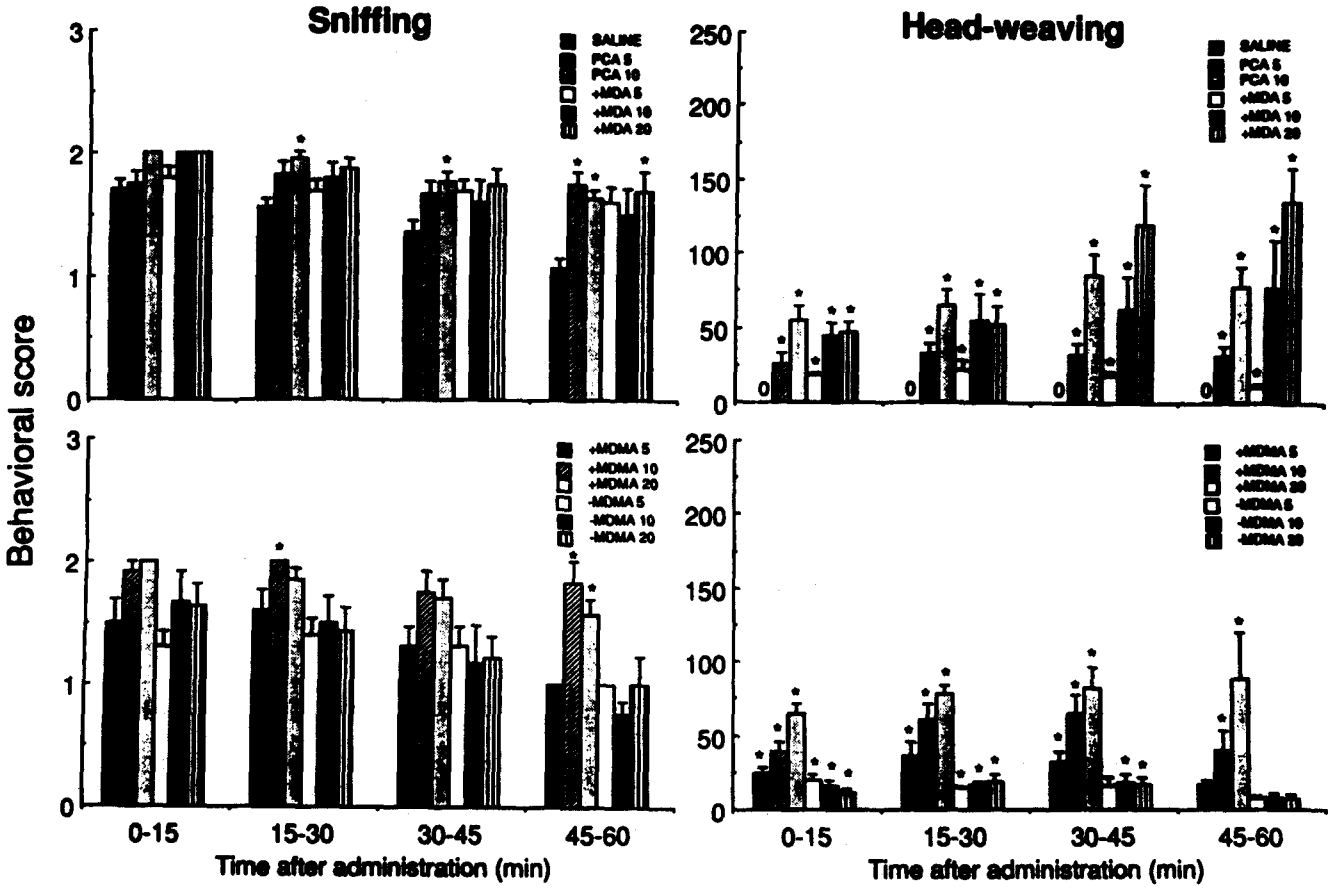

FIG. 2. Comparison of sniffing and head-weaving induced by *p*-chloroamphetamine (PCA), S(+)-MDMA, R(−)-MDMA and S(+)-MDA in rats. For further details see the legend in Fig. 1.

correlated with drug levels.

When examined within 1 hr after dosage S(+)-, R(−)-MDMA and S(+)-MDA produced stereotyped behavioral responses such as sniffing, head-weaving, backpedalling and turning (Figs. 1, 2). These responses were similar to those induced by *p*-chloroamphetamine, and by high doses of S(+)-amphetamine (7, 12, 19). During the first 15 min, significant differences were noted between the two MDMA enantiomers. At the doses tested, S(+)-MDMA produced 5 to 10 times more head-weaving, backpedalling and turning behaviors than the R(−) enantiomer (Figs. 1, 2). The desmethyl derivative of (+)MDMA, (+)MDA, was similar to MDMA in this response but appeared to be more potent and the response longer lasting. This stereotyped behavior is mediated by both DA and 5-HT systems. The 5-HT agonist, 5-methoxy-N,N-dimethyltryptamine, causes a complex behavioral syndrome that includes forepaw treading, hindlimb abduction, head-weaving, backpedalling and turning. The head-weaving and turning response is blocked by pretreatment with the DA agonist, apomorphine (19) indicating the involvement of this neurotransmitter system. Furthermore, the kappa opiate agonist, ethylketocyclazocine, which decreases DA release, antagonizes the backpedalling, head-weaving and turning responses but not the other behavioral responses (6). Forepaw treading and hindlimb abduction were not observed for the MDMA isomers in this dose range but could be discerned at high *p*-chloroamphetamine dosage.

At the neurochemical level, MDMA and MDA interact with the 5-HT and DA systems by causing an increased efflux or release of these transmitters (16). The release properties of the compounds exhibit enantiomeric differences, and the S(+) enantiomer is more potent in both 5-HT- and DA-releasing actions (6,16). MDMA also produces a reversible depletion of 5-HT and 5-HIAA (15,16). The reversible depletion of 5-HT is extensive at 10 mg/kg, with a minimum occurring a 3–6 hr after dosage. In contrast to the behavior effects, however, these depleting actions do not exhibit significant enantiomeric differences. The longer term, neurotoxic effect of MDMA on serotonergic nerve terminals that occurs one week after dosage was a property of the S(+) enantiomer of the drug (15). Thus, the stereoselective behavioral response noted here is more consistent with the releasing actions of MDMA and may also be associated with the development of its neurotoxicity.

Stereoselectivity in DA release (4) and in behavioral effects (10) has also been noted for amphetamine. However, the actions of amphetamine appear to be more selective to the DA system, whereas those of the methylenedioxy congeners are on both 5-HT and DA systems. The interactions of these methylenedioxy compounds with postsynaptic components of the 5-HT system also exhibit stereoselectivity, as the R(−) enantiomers of MDA and MDMA have affinities for the 5-HT_2, or ketanserine binding site that are 4 to 5 times greater than the (+) isomer (9). Thus, the presence of a methylenedioxy group on the phenyl ring of phenylisopropylamine results in a much more complex pharmacology involving the 5-HT system as well as the DA system. This 5-HT involvement exhibits a different stereoselectivity for pre- and postsynaptic events as the (−) enantiomer of MDMA is more

TABLE 1

COMPARISON OF WET-DOG SHAKE BEHAVIOR INDUCED BY *p*-CHLOROAMPHETAMINE (PCA), S(+)-MDMA, R(−)-MDMA AND S(+)-MDA IN RATS

Treatment		Time After Administration (min)			
Dose (mg/kg)		0–15	15–30	30–45	45–60
Saline		0	0	0	0
PCA	5	0.17 ± 0.17	3.17 ± 0.95*	5.50 ± 1.31*	5.00 ± 1.51*
PCA	10	0.64 ± 0.36	4.18 ± 1.28*	3.09 ± 0.90*	2.55 ± 1.15*
S(+)-MDMA	5	0.25 ± 0.25	0	0.13 ± 0.13	0.13 ± 0.13
	10	0	0.17 ± 0.17	0	0.17 ± 0.17
	20	0	0	0	0
R(−)-MDMA	5	0.25 ± 0.25	0.25 ± 0.25	0.13 ± 0.13	0
	10	0	0	0	0
	20	0	0	0	0
S(+)-MDA	5	0.13 ± 0.13	2.25 ± 1.01*	1.00 ± 0.38	0.13 ± 0.13
	10	1.40 ± 0.60	3.00 ± 0.71*	1.60 ± 0.93*	0.80 ± 0.58
	20	0.63 ± 0.32	1.88 ± 0.88*	0.25 ± 0.16	0.38 ± 0.38

Each value is the mean ± S.E.M. of 7–9 animals.
*$p<0.05$ vs. saline-treated group (Mann-Whitney U-test).

effective on the 5-HT_2 receptor, whereas the (+) enantiomer is more effective as a 5-HT releasing agent. As the (+) enantiomer was more potent in the behavioral effects studied here, they appear to be the result of presynaptic rather than postsynaptic events. Metabolism could also contribute to these differences between the two enantiomers. If there were enantiomeric differences in levels of the pharmacologically-active metabolites, MDA and the catecholamine, N-methyl-α-methyl dopamine, the overall response to the parent compounds would be different.

In these experiments, S(+)-MDA and *p*-chloroamphetamine produced wet-dog shake behavior but S(+)- and R(−)-MDMA produced very little (Table 1). Thus, MDMA may be pharmacologically different from MDA and *p*-chloroamphetamine, but further studies with these compounds are needed to elucidate the basis for the different behavioral patterns.

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