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Tolerance and Cross-Tolerance to 3,4-Methylenedioxymethamphetamine (MDMA), Methamphetamine and Methylenedioxyamphetamine

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ZACNY, J. P., R. M. VIRUS AND W. L. WOOLVERTON. *Tolerance and cross-tolerance to 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine and methylenedioxyamphetamine.* PHARMACOL BIOCHEM BEHAV **35**(3) 637–642, 1990.—The effects of (±)-3,4-methylenedioxymethamphetamine (MDMA) (0.62–20.0 mg/kg), (+)-methamphetamine (MA) (0.62–5.0 mg/kg) and (±)-3,4-methylenedioxyamphetamine (MDA) (0.62–5.0 mg/kg) on milk intake in rats were determined before and during a period of repeated daily administration of MDMA. Experimental sessions consisted of 15-min access to a sweetened milk solution each day, 5 days a week. After initial determination of the effects of MDMA, MA and MDA on milk intake, rats were injected daily with either MDMA (2.5–5.0 mg/kg) or saline. Two groups of rats were injected with MDMA, one group 15 min before, and the other group 15 min after the milk-drinking sessions. Two more groups of rats were injected with saline, one group 15 min before, and the other group 15 min after the sessions. During this repeated administration period the effects of MDMA, MA and MDA (injected 15 min prior to the session) were redetermined. In rats that had been injected with MDMA on a daily basis either before or after the milk-drinking sessions, the dose-response function of MDMA was shifted to the right, indicating that tolerance had developed. Cross-tolerance to MA appeared to develop only in the group of rats that had been injected with MDMA on a daily basis before the milk-drinking sessions. Cross-tolerance to MDA did not develop in any of the 4 groups of rats.

Tolerance Cross-tolerance Milk-drinking Rat Methylenedioxymethamphetamine Methamphetamine Methylenedioxyamphetamine

(±)-3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") is an amphetamine derivative recently listed by the Drug Enforcement Agency as a Schedule 1 drug. Currently, there is a great deal of interest in characterizing the behavioral effects of this drug because of its abuse potential and neurotoxicity. Several recent studies have examined the acute effects of MDMA on behavior in laboratory animals. In drug discrimination studies using monkeys, rats, and pigeons, MDMA has been shown to substitute for psychomotor stimulants such as *d*-amphetamine and *l*-cathinone as well as for several other drugs that are not psychomotor stimulants, i.e., fenfluramine and tetrahydro-β-carboline (3, 5, 8, 14). Interestingly, MDMA does not substitute for the hallucinogens, 1-(2,5-dimethoxy-4-methyl-phenyl)-2-aminopropane (DOM) and lysergic acid diethylamide (LSD) (6,12), which is consistent with the observation that MDMA does not produce hallucinations in humans (19). In animal self-administration studies, intravenous MDMA functions as a positive reinforcer in both rhesus monkeys and baboons (1,9). In addition, MDMA, like many other drugs of abuse, lowers the threshold for rewarding intracranial self-stimulation (7). MDMA has also been found to reduce response rate under both simple and complex operant schedules of reinforcement (4,21).

The effects of repeated administration of MDMA on behavior have not been examined. Accordingly, we examined the effects of a range of doses of MDMA on milk drinking in rats before and during repeated administration of MDMA. A shift to the right or left in the dose-response curves of MDMA would be indicative of tolerance or sensitization, respectively. In order to determine if environmental variables play a role in whether or how much tolerance or sensitization developed to MDMA (2,18), we repeat-

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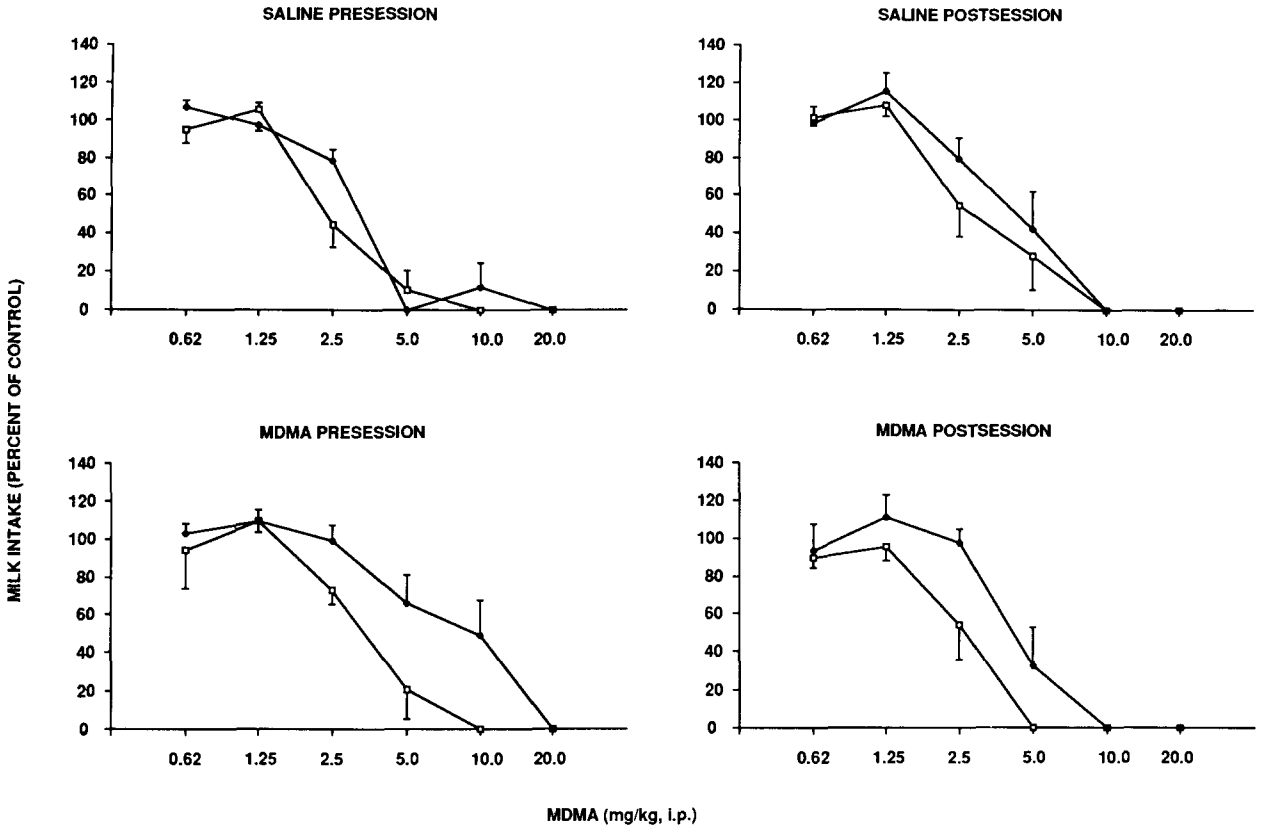

FIG. 1. Milk intake (mean ± SE) as a function of dose of MDMA for the dose-response functions determined before (open symbols) and during (solid symbols) the repeated administration period. Milk intake is expressed as a percentage of milk intake during test sessions in which saline was injected 15 min before the milk-drinking sessions. Mean absolute milk intake averaged across the two test sessions was 113.0 ± 3.3 ml/kg before, and 113.0 ± 4.9 ml/kg during the repeated administration periods, respectively. Two groups of rats were injected with saline during the repeated administration period, one group before (top left frame; N = 5) and the other group after (top right frame; N = 5), the milk-drinking session. Two groups of rats were injected with MDMA during the repeated administration period, one group 15 minutes before (bottom left frame, N = 6) and the other group 15 minutes after (bottom right frame; N = 5), the milk-drinking session. Doses of MDMA in the dose-response functions were injected 15 min before the milk-drinking session.

edly administered MDMA to one group of rats before the milk drinking session, and to another group of rats after the milk drinking session. Environmental variables (e.g., reinforcement loss) would be implicated in the development of tolerance if a greater degree of tolerance developed in the group of rats administered MDMA prior to, rather than after the milk-drinking session. In addition, dose-response functions were completed for two other structurally related amphetamine derivatives, methamphetamine (MA) and methylenedioxyamphetamine (MDA) before and during repeated MDMA administration to determine if there is cross-tolerance between MDMA, MA and MDA.

METHOD

Subjects and Apparatus

Twenty-one male Sprague-Dawley rats (Holtzman Co.; Madison, WI) weighing between 240 and 280 g prior to administration of test drugs were individually housed in stainless steel cages with water available ad lib except during the experimental sessions. Sweetened condensed milk (Borden's Co., Columbus, OH; 1:2, milk/tap water) was presented in 100-ml calibrated tubes attached centrally to the front of the cages. Supplemental feedings of 8 to 12 g of rat chow (4% Mouse and Rat Diet, Teklad Incorporated, Winfield, IA) were given approximately 4–5 hours after the sessions to maintain the rats throughout the experiment at 280 g (± 20 g). A 0700–2000 hr light-dark cycle was maintained in the colony room with a constant temperature of 22°C.

Procedure

Dose-response determinations. Experimental sessions consisted of a single 15-min presentation of 50 ml of milk at approximately the same time of day (1215 hr), Monday through Friday. After 19 sessions, at which point milk intake had stabilized (less than 10% variation in mean intake for three consecutive sessions), dose-response functions were determined for MDMA (0.62–20.0 mg/kg), MDA (0.62–5.0 mg/kg), and MA (0.62–5.0 mg/kg). Test doses were administered IP 15 min before the milk-drinking sessions on Tuesdays and Fridays. Order of drug and dose presentation was randomized (i.e., 1.25 mg/kg MA, 10 mg/kg MDMA, 1.25 mg/kg MDMA, 5.0 mg/kg MDA . . .), with all rats receiving the same drug treatment in a given test session. In two test sessions, rats were given injections of physiological saline. Milk intake data collected from these sessions served as control values to compare milk intake during test days in which

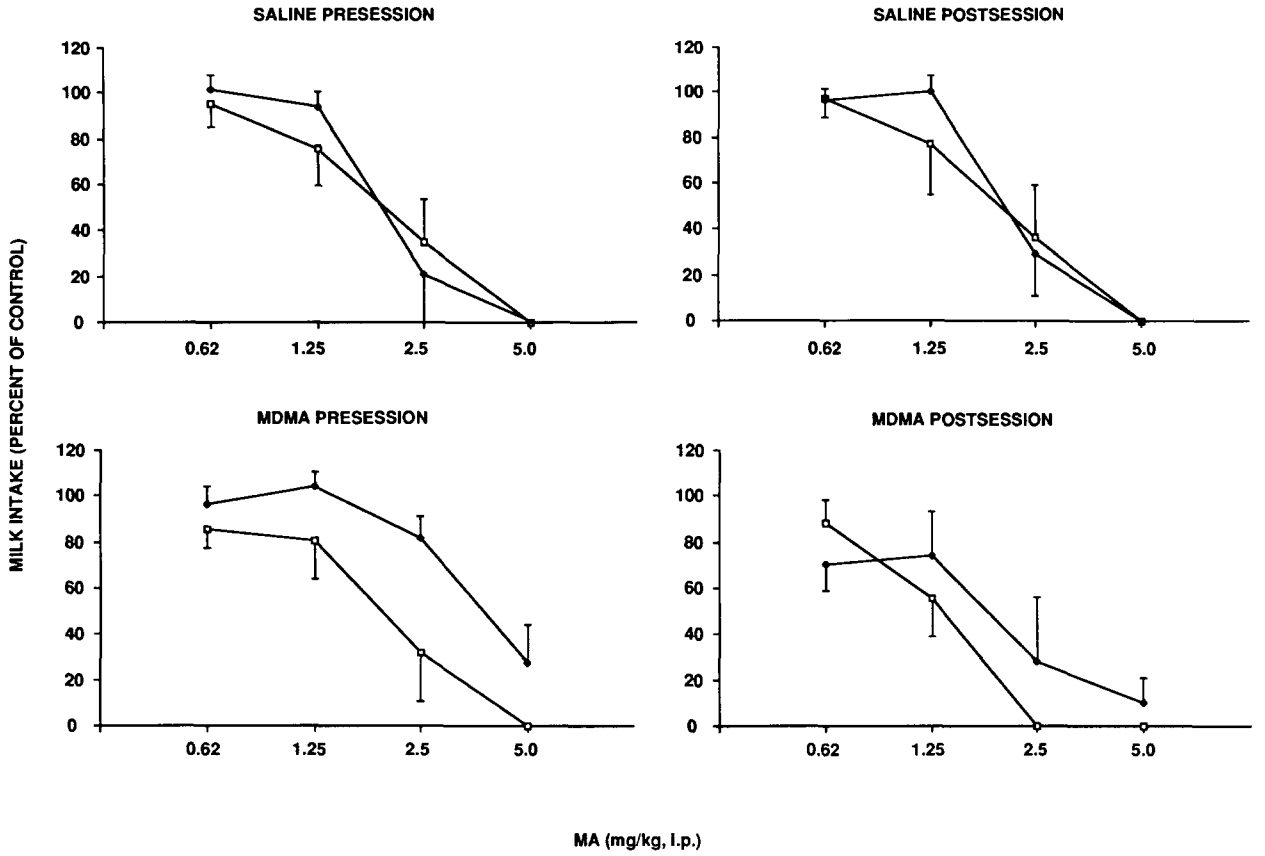

FIG. 2. Milk intake (mean ± SE) as a function of dose of MA for the dose-response functions determined before (open symbols) and during (solid symbols) the repeated administration period. Doses of MA in the dose-response functions were injected 15 min before the milk-drinking session. Other details as described in Fig. 1.

drug was injected. These saline test days occurred at the beginning and the middle of the series of dose-response function determinations.

Repeated administration regimen. After the initial dose-response determinations were completed for the three drugs, the rats were randomly assigned to one of four groups. Two groups of animals received daily (Monday–Friday) injections of physiological saline; one of the groups (N = 5) received saline 15 min before the milk-drinking sessions and the other group (N = 5) received saline 15 min after the sessions. The other two groups received daily (Monday–Friday) injections of MDMA; one of the groups (N = 6) received MDMA 15 min before the sessions and the other group (N = 5) received MDMA 15 min after the sessions. Initially the daily dose of MDMA was 2.5 mg/kg, a dose that decreased mean milk intake in the rats to less than 50% of control levels, but did not entirely eliminate intake during the initial MDMA dose-response determination. After 13 sessions, at which time tolerance had developed to 2.5 mg/kg MDMA in the group of rats injected with the drug prior to the session, the dose of MDMA which was administered on a daily basis was increased to 5.0 mg/kg.

Following 30 sessions of repeated administration of either MDMA or saline, dose-response functions for the three phenylisopropylamines were redetermined exactly as they were initially determined. On test days in which drug was given, rats did not receive their normal injections of either saline or MDMA. On test days in which saline was given, the rats that normally received MDMA injections either pre- or postsession were given 5.0 mg/kg of MDMA approximately four hours after the milk-drinking session in order to maintain the repeated MDMA administration regimen.

Drugs

(+)-Methamphetamine HCl (MA), (±)-methylenedioxyamphetamine HCl (MDA), and (±)-methylenedioxymethamphetamine HCl (MDMA) were provided by the National Institute on Drug Abuse. All drugs were dissolved in physiological saline and given in a volume of 1.0 ml/kg. Doses are expressed as the weight of the salt.

Data Analysis

Effects of the three drugs on milk intake are expressed as a percentage of milk intake from test sessions in which physiological saline was given prior to the session. ED_{50} values with 95% confidence limits were determined for the visibly linear portion of each dose-response function in each of the four groups by the method of least squares linear regression. Initial and redetermined dose-response functions were considered significantly different if the ED_{50} for each function was not within the 95% confidence interval of the other. In each group, milk intake during the repeated administration period was expressed as a percentage of

TABLE 1

ED_{50} VALUES AND 95% CONFIDENCE INTERVALS OF MDMA, MDA, AND MA FOR DOSE-RESPONSE FUNCTIONS DETERMINED BEFORE AND DURING A PERIOD OF REPEATED ADMINISTRATION OF EITHER SALINE OR MDMA

	Before (mg/kg)	During (mg/kg)
MDMA		
Saline-Presession	2.82 (2.24–3.47)	2.69 (2.46–2.95)
Saline-Postsession	3.39 (2.63–4.37)	4.17 (3.39–5.37)
MDMA-Presession	3.63 (3.09–4.27)	7.24* (5.62–10.23)
MDMA-Postsession	2.51 (2.04–3.09)	4.27* (3.39–5.37)
MA		
Saline-Presession	1.82 (1.38–2.40)	2.09 (1.55–2.63)
Saline-Postsession	1.86 (1.32–2.69)	2.29 (1.82—2.75)
MDMA-Presession	2.04 (1.26–2.75)	3.63* (2.81–5.62)
MDMA-Postsession	1.20 (0.98–1.40)	1.91 (0.14–3.39)
MDA		
Saline-Presession	2.46 (2.00–3.09)	2.63 (2.40–2.95)
Saline-Postsession	2.40 (2.00–2.88)	2.29 (1.91–2.82)
MDMA-Presession	2.88 (2.34–3.55)	3.24 (2.69–4.27)
MDMA-Postsession	2.00 (1.62–2.57)	2.24 (1.74–3.02)

*Ninety-five percent confidence intervals of the two dose-response functions do not overlap.

milk intake measured during the 3 sessions with no pretreatment immediately prior to the repeated administration period.

RESULTS

Dose-Response Determinations

MDMA (0.62–10.0 mg/kg) decreased milk intake in all four groups in a dose-dependent fashion both during the initial and redetermined dose-response functions (Fig. 1). The 20.0 mg/kg dose of MDMA suppressed milk intake in all rats. Initial and redetermined dose-response functions differed significantly from each other in the drug groups, but not in the saline groups: the 95% confidence limits around the ED_{50} of the initial dose-response function and the redetermined dose-response function did not overlap in the MDMA-Presession and MDMA-Postsession group, but did overlap in the groups that received repeated administration of saline (Table 1). Figure 1 reveals that when the effects of MDMA were redetermined during repeated administration, dose-response functions were shifted to the right in the drug groups (bottom panels) but not in the saline groups (top panels).

MA (0.62–5.0 mg/kg) decreased milk intake in a dose-dependent fashion both before and during repeated MDMA administration (Fig. 2). Initial and redetermined dose-response functions differed significantly from each other only in the MDMA Presession group: the 95% confidence limits around the ED_{50} of the initial dose-response function and the redetermined dose-response function did not overlap in the MDMA-Presession group, but did overlap in the other three groups (Table 1). When the effects of MA were redetermined during the repeated administration period, dose-response functions shifted to the right in the MDMA-Presession group (bottom left panel). Although it appears that there also was a shift to the right in the redetermined dose-response function in the MDMA Postsession group (bottom right panel), this group function is not representative of individual subject milk-drinking. Four of the five rats in this group did not exhibit tolerance to MA upon redetermination of the dose-response function, i.e., they did not drink after administration of the 2.5 mg/kg MA dose.

MDA (0.62–5.0 mg/kg) decreased milk intake in a dose-dependent fashion both before and during the repeated adminstration of MDMA (Fig. 3). Initial and redetermined dose-response functions in each of the four groups did not differ significantly from each other: the 95% confidence limits around the ED_{50} of the initial dose-response function and the redetermined dose-response function overlapped in all four groups (Table 1). It is also apparent from Fig. 3 that when the effects of MDA were redetermined during repeated administration, dose-response functions did not shift in any of the four groups.

Milk Intake During Repeated Administration

The two groups of rats that were injected with saline prior to or after the milk drinking session generally maintained milk drinking levels observed during baseline throughout the experiment. Milk intake in the MDMA-Presession group was initially decreased by 2.5 mg/kg MDMA to about 65% of baseline levels. By the 13th session of repeated injections, milk intake had recovered to within 90% of baseline levels. After initiating the higher dose injection regimen, i.e., 5.0 mg/kg, milk intake levels were reduced to below 70% of baseline levels and remained there until the end of the repeated administration period. The MDMA-Postsession group was not affected initially by injections of MDMA, but four sessions after the dose of MDMA was increased to 5.0 mg/kg, milk intake began to decline, and by the 13th session of the higher dose regimen, was at 70% of baseline levels. Milk intake remained at this level for approximately 21 additional sessions and then increased again to 90% of baseline levels by the end of the repeated administration period.

DISCUSSION

The purpose of the present study was to determine whether repeated administration of MDMA produced subsequent tolerance to MDMA and cross-tolerance to two structurally related phenylisopropylamines, MA and MDA, using a milk-drinking procedure. Tolerance to MDMA was observed in those rats injected with MDMA daily either before or after the milk-drinking session. Cross-tolerance to MA appeared to have developed only in those rats who were injected with MDMA daily before the milk-drinking session. No cross-tolerance to MDA was noted.

The fact that tolerance to MDMA occurred not only in those rats which were injected with MDMA on a daily basis before milk-drinking sessions, but also in those rats which were injected with MDMA after the milk-drinking sessions suggests that tolerance to MDMA includes a pharmacological component. It should be acknowledged that an even greater degree of pharmacological

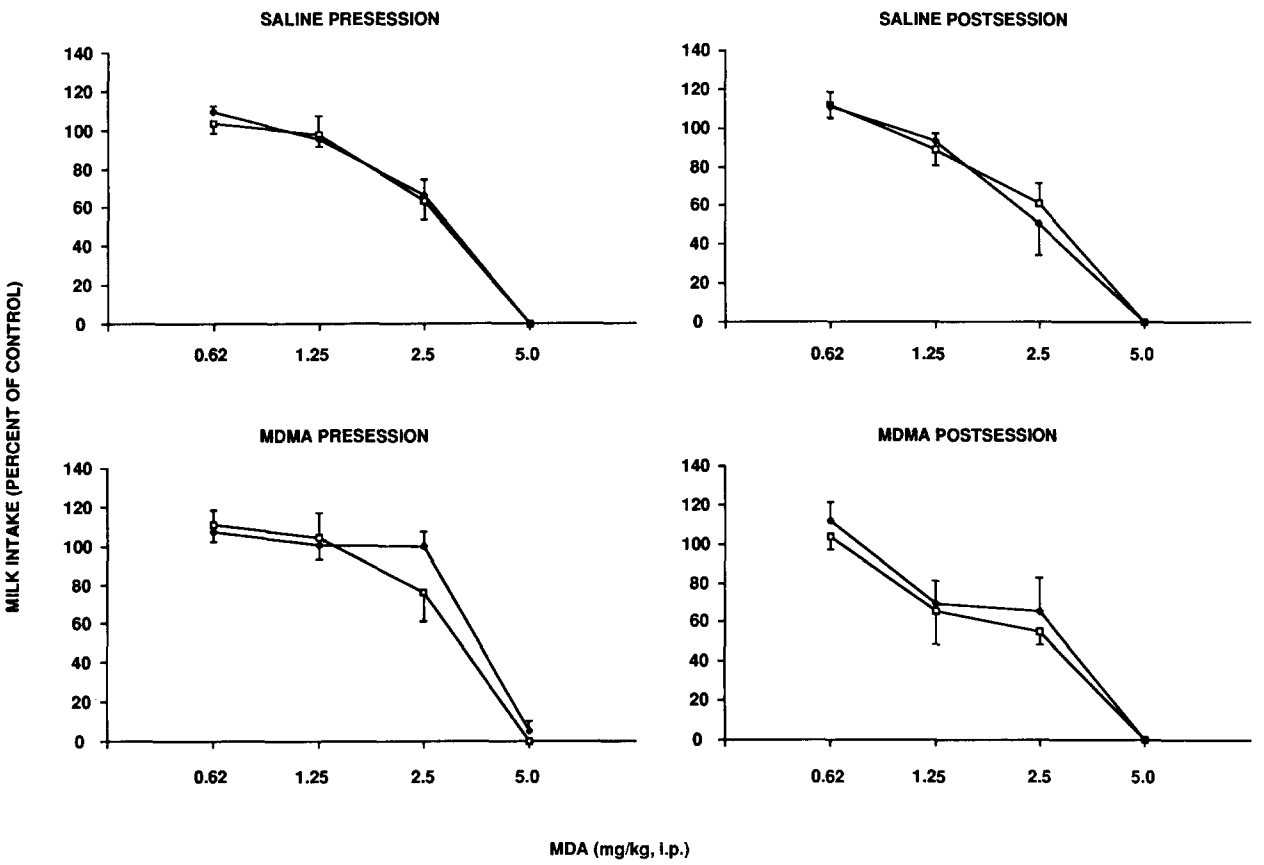

FIG. 3. Milk intake (mean ± SE) as a function of dose of MDA for the dose-response functions determined before (open symbols) and during (solid symbols) the repeated administration period. Doses of MDA in the dose-response functions were injected 15 min before the milk-drinking session. Other details as described in Fig. 1.

tolerance to MDMA might have developed had a 7-day/week chronic MDMA regimen rather than the 5-day/week chronic regimen been used. It is also conceivable that the weekly two-day drug holidays (Saturday and Sunday) during the chronic MDMA regimen may have reduced the amount of tolerance to MDMA in the early part of the week in the MDMA-Presession and Postsession groups. To investigate whether there was a within-week pattern of tolerance to MDMA, mean milk intake from the first control day of the week (Monday) was compared to mean milk intake from the last control day of the week (Thursday) in the MDMA-Presession group during the chronic MDMA regimen: there were no significant differences in milk intake between these two timepoints, $t(5) = 1.5$, ns. The fact that there did not appear to be a within-week pattern of tolerance to MDMA suggests that milk intake from the two test days (Tuesday and Friday) were not confounded by differential degrees of tolerance to MDMA preceding the test days.

It is difficult to explain why cross-tolerance between MDMA and MA was observed only in the group of rats who were injected with MDMA before the milk-drinking sessions. Lack of cross-tolerance in the MDMA-Postsession group suggests that behavioral factors are involved in mediating cross-tolerance to MA, yet behavioral factors did not appear to be a major factor in the development of tolerance to MDMA in this group. Certainly the two compounds are related pharmacologically: MA and MDMA are structurally related and the mechanisms of action for MA and MDMA appear to involve both serotonin and catecholamines (15–17). Further research is needed to determine the mechanism(s) of action responsible for mediating cross-tolerance between MDMA and MA.

Cross-tolerance was not observed between MDMA and MDA. This is surprising, given that MDA is reportedly one of the metabolites of MDMA in rats (10). In addition, both drugs are equipotent releasers of 5-HT, DA and NE (17,20). There are differences between the behavioral effects of the two compounds, though, which suggests that the mechanisms of action of MDMA and MDA may not be identical. Firstly, MDA, but not MDMA, substitutes for the hallucinogens, DOM and LSD, in drug discrimination studies (6,12). In addition, DOM substitutes for MDA, but not MDMA (5,13). Secondly, the rate-reducing effects of MDMA and MDA are antagonized by different monoamine antagonists (11). In a multiple schedule of food reinforcement, metergoline and ketanserin, two 5-HT antagonists, blocked the rate-reducing effects of MDA, but not of MDMA. Prazosin, a NE antagonist, blocked the rate-reducing effects of MDMA, but not of MDA.

In summary, tolerance to the disruptive effects of MDMA on milk drinking occurred in rats that were given daily injections of 2.5 and 5.0 mg/kg of MDMA, regardless of whether the drug was injected before or after the milk-drinking session. Cross-tolerance to MA occurred in the MDMA-Presession group, but surprisingly not in the MDMA-Postsession group. Also surprising was the fact that cross-tolerance to MDA did not occur in any of the four

groups of rats. More studies need to be conducted with MDMA, MA and MDA which utilize other tolerance assessment procedures before any firm conclusions can be drawn regarding the extent to which cross-tolerance exists between these three compounds.

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REFERENCES

1. Beardsley, P. M.; Balster, R. L.; Harris, L. S. Self-administration of methylenedioxymethamphetamine (MDMA) by rhesus monkeys. Drug Alcohol Depend. 18:149–158; 1986.
2. Carlton, P. L.; Wolgin, D. L. Contingent tolerance to the anorexigenic effects of amphetamine. Physiol. Behav. 7:221–223; 1971.
3. Evans, S. M.; Johanson, C. E. Discriminative stimulus properties of (±)-methylenedioxymethylamphetamine and (±)-methylenedioxyamphetamine in pigeons. Drug Alcohol Depend. 18:159–164; 1986.
4. Glennon, R. A.; Little, P. J.; Rosecrans, J. A.; Yousif, M. The effects of MDMA ("Ecstasy") and its optical isomers on schedule-controlled responding in mice. Pharmacol. Biochem. Behav. 26:425–426; 1987.
5. Glennon, R. A.; Young, R. Further investigation of the discriminative stimulus properties of MDA. Pharmacol. Biochem. Behav. 20: 501–505; 1984.
6. Glennon, R. A.; Young, R.; Rosecrans, J. A.; Anderson, G. M. Discriminative stimulus properties of MDA analogues. Biol. Psychiatry 17:807–814; 1982.
7. Hubner, C. B.; Bird, M.; Rassnick, S.; Kornetsky, C. The threshold lowering effects of MDMA (ecstasy) on brain-stimulation reward. Psychopharmacology (Berlin) 95:49–51; 1988.
8. Kamien, J. B.; Johanson, C. E.; Schuster, C. R.; Woolverton, W. L. The effects of (±)-methylenedioxyamphetamine and (±)-methylenedioxymethylamphetamine in monkeys trained to discriminate (+)-amphetamine from saline. Drug Alcohol Depend. 18:139–147; 1986.
9. Lamb, R. J.; Griffiths, R. R. Self-injection of *d,l*-3,4-methylenedioxymethamphetamine (MDMA) in the baboon. Psychopharmacology (Berlin) 91:268–272; 1987.
10. Lim, H. K.; Foltz, R. L. Metabolism of 3,4-methylenedioxymethamphetamine (MDMA) in rat. FASEB Abstracts 2:A-1060; 1988.
11. Nader, M. A.; Hoffmann, S. M.; Barrett, J. E. Behavioral effects of 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA) in the pigeon: interactions with noradrenergic and serotonergic systems. Psychopharmacology (Berlin) 98: 183–188; 1989.
12. Nichols, D. E.; Hoffman, A. J.; Oberlender, R. A.; Jacob, P., III; Shulgin, A. T. Derivatives of 1-(1,3-benzodioxol-5-yl)-2-butanamine: Representatives of a novel therapeutic class. J. Med. Chem. 29:2009; 1986.
13. Oberlender, R.; Nichols, D. E. Drug discrimination studies with MDMA and amphetamine. Psychopharmacology (Berlin) 95:71–76; 1988.
14. Schechter, M. D. Discriminative profile of MDMA. Pharmacol. Biochem. Behav. 24:1533–1537; 1986.
15. Schmidt, C. J.; Gibb, J. W. Role of the dopamine uptake carrier in the neurochemical response to methamphetamine: Effects of amfonelic acid. Eur. J. Pharmacol. 109:73–80; 1985.
16. Schmidt, C. J.; Gibb, J. W. Role of the serotonin uptake carrier in the neurochemical response to methamphetamine: Effects of citalopram and chlorimipramine. Neurochem. Res. 10:637–648; 1985.
17. Schmidt, C. J.; Levin, J. A.; Lovenberg, W. In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. Biochem. Pharmacol. 36: 747–755; 1987.
18. Schuster, C. R.; Dockens, W. S.; Woods, J. H. Behavioral variables affecting the development of amphetamine tolerance. Psychopharmacologia 9:170–182; 1966.
19. Shulgin, A. T.; Nichols, D. E. Characterization of three new psychotomimetics. In: Stillman, R. C.; Willete, R. E., eds. The psychopharmacology of hallucinogens. New York: Pergamon Press; 1978:74–83.
20. Steele, T. D.; Nichols, D. E.; Yim, G. K. W. Stereochemical effects of 3,4-methylenedioxymethamphetamine (MDMA) and related amphetamine derivatives on inhibition of uptake of [^{3}H]monoamines into synaptosomes from different regions of rat brain. Biochem. Pharmacol. 36:2297–2303; 1987.
21. Thompson, D. M.; Winsauer, P. J.; Mastropaolo, J. Effects of phencyclidine, ketamine and MDMA on complex operant behavior in monkeys. Pharmacol. Biochem. Behav. 26:401–405; 1987.