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MDMA (Ecstasy) Substitutes for the Ethanol Discriminative Cue in HAD But Not LAD Rats

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MEEHAN, S. M., T. L. GORDON AND M. D. SCHECHTER. *MDMA (Ecstasy) substitutes for the ethanol discriminative cue in HAD but not LAD rats.* ALCOHOL 12(6) 569–572, 1995.—Selectively bred high- and low-alcohol-drinking (HAD/LAD) rats were trained to discriminate the interoceptive stimuli produced by IP-administered 600 mg/kg ethanol (10% w/v) in a two-lever, food-motivated operant task. Once criterion discrimination was attained, animals were tested with 3.0, 1.5, 1.0, and 0.5 mg/kg MDMA. Although no differences in alcohol discrimination were observed between the HAD and LAD animals, the HAD line was significantly more sensitive than the LAD line to the effects of MDMA. These results provide additional information to the growing body of evidence suggesting serotonergic mediation of some of the behavioral effects of ethanol.

Drug discrimination Ethanol HAD/LAD MDMA Serotonin

NUMEROUS studies have indicated that central serotonergic systems play a role in the mediation of ethanol consumption and preference in both animal (9,13,15) and human (11) subjects. The Fawn-Hooded (FH) rat strain possesses a genetically linked serotonergic storage abnormality and they display a complete absence or greatly diminished ability to bind [^{3}H]imipramine in both platelets and brain tissue (1,4). Recently, it has been shown that the Wistar (W)-derived FH strain exhibits a high preference for alcohol relative to W and Sprague–Dawley (SD) strains (14). Similarly, animals that have been selectively bred for ethanol preference, specifically the alcohol-preferring (P) and high-alcohol-drinking (HAD) lines of rat, exhibit lower levels of serotonin (5-HT) and the serotonergic metabolite 5-hydroxyindolacetic acid (5-HIAA), as well as a greater number of 5-HT binding sites in hippocampus and frontal cortex, compared to their ethanol-nonpreferring (NP) and low-alcohol-drinking (LAD) counterparts (10,21).

The high level of ethanol consumption observed in both P and FH rats has recently been reported (12) to be attenuated, without affecting ethanol pharmacokinetics, by administration of the psychoactive drug 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy"; "XTC"). However, at present, there are no data examining the effects of MDMA on ethanol intake in the HAD/LAD lines.

The drug discrimination paradigm requires an animal to produce an operant response, typically the press of one lever, following administration of a drug and to press a second, alternative lever after administration of the vehicle. This technique has been used in at least 1675 reports (19) to characterize the psychoactive properties of drugs with ethanol being the first drug so determined (3). Furthermore, it has been shown that the selective 5-HT$_1$B agonist TFMPP substitutes for the ethanol cue in heterogeneously bred animals (18) and attenuates ethanol intake in P rats (9). Based on this notion, the current study sought to examine the degree to which MDMA might produce internal cues similar to ethanol in the ethanol-trained HAD and LAD line of rats.

METHOD

Subjects

Nine HAD-2 and 10 LAD-2 male rats were the subjects used and were individually housed in standard hanging wire cages in a climate-controlled Vivarium facility kept at an ambient temperature of 20–22°C on a 12 : 12 light : dark cycle (light 0600–1800 h). Although water was available ad lib, restricted access to commercial rat chow after each session allowed maintenance of rat weights at 85% ± 5% of their free-feeding weights. This procedure facilitated motivation of operant performance for food reward.

Apparatus

The experimental environment consisted of 12 standard rodent operant test chambers (Lafayette Instrument Corp.,

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Lafayette, IN) with each chamber equipped with two operant levers and a food receptacle at an equal distance between the levers. Solid-state equipment (LVB Corp., Lehigh Valley, PA), used to control and record the sessions, was located in an adjacent room.

Lever Pressing and Discrimination Training

The food-deprived animals were trained to press one lever while in the drug state and the alternative lever following administration of the distilled water vehicle. Training sessions were conducted once a day, 5 days a week, with one lever in each chamber designated as the ethanol lever and the second lever designated as the vehicle lever. To preclude any possible position preference, half the rats were required to press the ethanol lever at the right of the food receptacle, whereas for the other five animals, the ethanol lever was to the left of the receptacle. The other lever was designated as the vehicle lever. Initially, all animals were trained to respond on the vehicle lever 10 min after the IP administration of 5 ml/kg distilled water on a fixed ratio (FR) schedule of 1 [i.e., one response resulted in one (45-mg Noyes food pellet) reinforcement]. During 10 consecutive training sessions, the FR schedule was gradually incremented to an FR10 in that 10 responses on the vehicle lever yielded one food reinforcement. The animal was removed from the operant chamber and returned to the home cage after receiving 40 reinforcements on each FR schedule.

Once an FR10 was established on the vehicle lever, training began on the opposite lever (10 min) following the injection (IP) of an equal volume containing 600 mg/ml (10% w/v) ethanol. Rats subsequently were reinforced only for responding on the ethanol lever and, as with previous training after vehicle, the initial reinforcement schedule of FR1 was gradually incremented to FR10; this was accomplished over a period of seven daily sessions.

The rats were considered to be trained to lever press after FR10 responding was established on both levers. Discrimination training was subsequently initiated 10 min after the daily administration of either the vehicle or ethanol. Animals received vehicle (V) or ethanol (E) according to a 2-week, repeating, administration schedule: E,V,V,E,E; V,E,E,V,V. The first lever upon which 10 responses were accumulated at the beginning of each session was considered the "selected" lever for that daily session. At the time of the 10th response, presses on both the selected and nonselected levers were recorded. The session was continued, regardless of the correctness of the selected lever, until 400 responses were made on the correct lever for that session and, therefore, 40 reinforcements (on the FR10 schedule) were received. The ethanol and vehicle training regimen continued for 12 weeks to allow all of the animals to attain a criterion level of discriminative performance designated as correctly choosing the lever appropriate for the injection received (condition imposed) in 8 of 10 consecutive daily training sessions, twice. The sessions-to-criterion (STC 1) measurement indicates the first session of 10 consecutive daily sessions in which eight first-choice lever selections were initially made. The STC 2 relates to the first session of a second set of 8 of 10 correct consecutive lever selections. This 80% performance level was required for all subjects before dose-response testing was initiated.

Dose-Response Tests

Once the discriminative criterion was attained by all animals, the discriminative training regimen was limited to alternating days to ensure maintenance of the ethanol-vehicle discrimination. On intervening days, rats were tested with three doses of ethanol (900, 450, and 300 mg/kg) differing from the 600 mg/kg training dose with each dose tested twice, once following a drug maintenance session and once following a vehicle session. This counterbalanced design was employed to control for any possible residual influences from the previous day's maintenance session. Once 10 reponses were accumulated on either lever, the rat was immediately removed, without receiving reinforcement, and placed into its home cage. This intended to preclude reinforcement/training at a dose different from the ethanol training dose.

Generalization to MDMA

To assess the potential transfer (generalization) of the ethanol cue to MDMA, animals were tested with MDMA (3.0, 1.5, 1.0, and 0.5 mg/kg, SC) in sessions interspersed with maintenance ethanol or vehicle sessions. Twenty minutes following MDMA administration, rats were placed into the experimental chamber and were allowed to lever press until 10 responses were made on either of the two levers. When 10 responses had accumulated on either lever, the animal was immediately removed from the experimental chamber, without receiving reinforcement. The first lever pressed 10 times was, thus, designated as the "selected" lever. Each MDMA dose was administered in a random order on two occasions with each test session preceded by one vehicle and one ethanol maintenance session. In this way, animals' experience on test days was counterbalanced with respect to any possible aftereffects that may have been produced by the previous day's training condition.

Measurements and Statistical Analysis

The percentage of rats selecting (accumulating 10 presses first upon) the ethanol-appropriate lever constituted the quantal discrimination measurement. The subjects were required to maintain a minimum of 80% ethanol-appropriate lever selections during drug maintenance sessions and were permitted a maximum of 20% ethanol-appropriate lever selections after vehicle injections for any 10 consecutive maintenance sessions. This established that an animal recognized the ethanol or vehicle cue at an 80% accuracy rate during maintenance and all animals met this performance criterion during the course of the entire study. Based on this maintenance criterion, it was determined ad hoc that, in substitution tests, MDMA needed to produce lever selection equal to or greater than 80% ethanol appropriate to be considered to generalize for ethanol.

In addition to the quantal measurements, quantitative discrimination measurements were also calculated. The quantitative measurement is the number of responses on the ethanol lever divided by the sum of the responses on the ethanol and vehicle lever at the time that 10 responses are accumulated on either lever. This fraction is expressed as a percentage and, unlike the all-or-none quantal measurement, encompasses responses on both the selected and unselected levers, thus providing an assessment of the magnitude, as well as the direction, of lever preference.

The dose-response quantal data were subjected to the Litchfield-Wilcoxon (7) analysis that employs log-dose vs. probit measurements. A computer-generated formulation (20) of the procedure yielded an ED_{50} value (with 95% confidence limits) for the dose-response curves of ethanol and MDMA. This analysis (7) also allows for tests of parallelism between dose-response curves, as well as for potency differences between drugs.

RESULTS

Training and Dose-Response Testing

The discrimination training record for HAD and LAD animals indicated equivalent acquisition rates of the ethanol discrimination with a mean ± SD of 16.9 ± 8.8 training sessions to the first criterion (i.e., STC1) for the HAD animals and 22.4 ± 10.9 for the LAD rats. The second criterion (STC2) was achieved in a mean of 35.3 ± 10.9 training sessions for the HAD rats and 37.2 ± 13.7 for the LAD rats. Thus, all rats learned to discriminate ethanol from its vehicle after 60 sessions; 30 with ethanol and 30 with vehicle.

The dose-response data from these animals are presented in Table 1. Decreasing doses of ethanol (450 and 300 mg/kg) produced concommitant reductions in lever-appropriate responding in both the HAD and LAD animals, whereas increasing the dose of ethanol (900 mg/kg) in both lines enhanced ethanol-appropriate responding beyond that seen after the maintenance dose of 600 mg/kg only in the HAD rats. The ED_{50} values, calculated using the Litchfield-Wilcoxon methodology (7), equaled 324.8 mg/kg with a 95% confidence interval of 246.0–428.9 mg/kg in the HAD rats and 281.7 mg/kg in the LAD rats with a 95% confidence interval of 187.2–423.9 mg/kg. The overlap in the confidence levels for the ED_{50} values suggests that there was no difference in sensitivity to ethanol effects between the HAD and LAD animals.

Agonism Studies

The results of dose-response generalization tests with MDMA are shown in Table 2. MDMA produced generalization (defined as 80% or better quantal responding) to the ethanol cue in the HAD animals at the highest dose employed (i.e., 3.0 mg/kg MDMA) whereas no generalization was found in LAD animals tested with MDMA. The ED_{50} value for MDMA was calculated to be 0.61 mg/kg with a 95% confidence limit of 0.29–1.24 mg/kg in the HAD rats. A test of parallelism (7) conducted on the data obtained from the HAD animals revealed no difference between the dose-response slopes of MDMA and ethanol (calculated $t = 2.56 <$ critical $t = 3.18$). Analysis of the potency ratio between MDMA and ethanol was significant, indicating greater potency of MDMA relative to ethanol.

TABLE 1

DOSE-RESPONSE DISCRIMINATIVE PERFORMANCE IN HAD AND LAD RATS TRAINED TO DISCRIMINATE 600 mg/kg (10% w/v) ETHANOL IP ADMINISTERED 10 MIN BEFORE TESTING

Dose ethanol (mg/kg)	Quantal	Quantitative (SD)
HAD rats (n = 9)		
900	94.5	77.9 (3.1)
600	88.9	73.4 (7.6)
450	66.7	62.7 (2.7)
300	44.5	51.6 (7.2)
ED_{50} (95% confidence limits): 324.8 (246.0–428.9) mg/kg		
LAD rats (n = 10)		
900	75.0	61.5 (2.1)
600	85.0	73.0 (3.0)
450	75.0	64.4 (5.2)
300	40.0	51.7 (3.7)
ED_{50} (95% confidence limits): 281.7 (187.2–423.9) mg/kg		

TABLE 2

DOSE-RESPONSE RELATIONSHIPS AFTER MDMA ADMINISTRATION IN HAD AND LAD RATS TRAINED TO DISCRIMINATE 600 mg/kg ETHANOL

Dose MDMA (mg/kg)	Quantal	Quantitative (SD)
HAD rats		
3.0	85.70	73.55 (9.8)
1.5	50.00	55.75 (5.8)
1.0	72.20	60.24 (14.4)
0.5	33.00	41.94 (1.3)
ED_{50} (95% confidence limits): 0.61 (0.29–1.24) mg/kg		
LAD rats		
3.0	31.58	41.41 (9.9)
1.5	30.00	31.32 (1.0)
1.0	16.66	27.16 (7.8)
0.5	30.00	34.04 (17.9)

DISCUSSION

The current data indicate that, whereas the HAD and LAD lines of rat have been selectively bred based upon differential levels of independent ingestion of ethanol, there is no apparent difference between the two lines in their ability to learn ethanol drug discrimination. Indeed, both the HAD and LAD animals learned the ethanol discrimination approximately at the same rate and exhibited similar ED_{50} values, suggesting equivalent sensitivity to the ethanol cue. These findings replicate those obtained by Krimmer and Schechter (6) and are comparable to those obtained with P and NP animals in our laboratory in that the P/NP animals also failed to exhibit differences in learning rates or ethanol sensitivity (5). It is plausable that the amount of training required in the drug discrimination task is so extensive as to essentially obscure subtle differences in ethanol sensitivity. However, additional data from our laboratory have shown that Fawn-Hooded rats exhibit a marked impairment in the acquisition of the ethanol discrimination and are much less sensitive to ethanol relative to Sprague-Dawley controls (17).

MDMA has been shown to have both serotonergic and dopaminergic agonist properties with predominant serotonergic action occurring approximately 20 min postadministration and peak dopaminergic activity occurring 105 min after adminstration (16). The present data in HAD rats, showing generalization of MDMA to the ethanol cue at 20 min postadministration, support the notion that serotonergic activation is an important component of the internal state produced by ethanol (9,13,15). Furthermore, the LAD animals trained on ethanol failed to show generalization with MDMA, reinforcing the premise that the differences in central serotonergic efficacy between the two strains may mediate their differential alcohol preference. Previous research indicates that there is greater 5-HT_1A (22) but lesser 5-HT_2 (8) receptor density in the P line than in the NP line, suggesting the former site as more appropriate for ethanol discrimination (18). Whether MDMA will alter ethanol ingestive behavior in HAD rats, as has been shown in FH and P rats (12), as well as in the Sprague-Dawley line (2), remains to be determined.

REFERENCES

1. Arora, R. C.; Tong, C.; Jackman, H. L.; Stoff, D.; Meltzer, H. Y. Serotonin uptake and imipramine binding in blood platelets and brains of Fawn-Hooded and Sprague-Dawley rats. Life Sci. 33:437–442; 1983.
2. Bilsky, E. J.; Hue, Y. Z.; Hubbell, C. L.; Reid, L. D. Methylenedioxymethamphetamine's capacity to establish place preference and modify intake of an alcoholic beverage. Pharmacol. Biochem. Behav. 37:633–638; 1990.
3. Conger, J. C. The effects of alcohol on conflict behavior in the albino rat. Q. J. Stud. Alcohol 12:1–29; 1951.
4. Dumbrille-Ross, A.; Tang, S. W. Absence of high-affinity [^{3}H] imipramine binding in platelets and cerebral cortex of Fawn Hooded rats. Eur. J. Pharmacol. 72:137–138; 1981.
5. Gordon, T. L.; Meehan, S. M.; Schechter, M. D. P and NP rats respond differently to the discriminative stimulus effects of nicotine. Pharmacol. Biochem. Behav. 45:305–308; 1993.
6. Krimmer E. C.; Schechter, M. D. HAD and LAD rats respond differently to stimulating effects but not discriminative effects of ethanol. Alcohol 9:71–74; 1991.
7. Litchfield, J. T.; Wilcoxon, F. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96:99–106; 1949.
8. McBride, W. J.; Chernet, E.; Rabold, J. A.; Lumeng, L.; Li, T-.K. Serotonin$_2$ receptors in the CNS of alcohol-preferring and -nonpreferring rats. Pharmacol. Biochem. Behav. 46:631–636; 1993.
9. McBride, W. J.; Murphy, J. M.; Lumeng, L.; Li, T.-K. Serotonin and ethanol preference. In: Galanter, M., ed. Recent developments in alcoholism. Vol. 7. New York: Plenum Press; 1989:187–209.
10. Murphy, J. M.; McBride, W. J.; Lumeng, L.; Li, T.-K. Contents of monoamines in forebrain regions of alcohol preferring (P) and nonpreferring (NP) lines of rats. Pharmacol. Biochem. Behav. 36:389–392; 1987.
11. Naranjo, C. A.; Sellers, E. M.; Lawrin, M. O. Modulation of ethanol intake by serotonin uptake inhibitors. J. Clin. Psychiatry 47(Suppl.):16–22; 1986.
12. Rezvani, A. H.; Garges, P. L.; Miller, D. B.; Gordon, C. J. Attenuation of alcohol consumption by MDMA (ecstasy) in two strains of alcohol-preferring rats. Pharmacol. Biochem. Behav. 43:103–110; 1992.
13. Rezvani, A. H.; Janowsky, D. S. Decreased alcohol consumption by verapamil in alcohol preferring rats. Prog. Neuropsychopharmacol. Biol. Psychiatry 14:623–631; 1990.
14. Rezvani, A. J.; Overstreet, D. H.; Janowsky, D. S. Genetic serotonin deficiency and alcohol preference in the Fawn Hooded rat. Alcohol 25:573–575; 1990.
15. Rezvani, A. H.; Overstreet, D. H.; Janowsky, D. S. Drug-induced reductions in ethanol intake in alcohol preferring and Fawn-Hooded rats. Alcohol Alcohol. 1(Suppl.):433–437; 1991.
16. Schechter, M. D. Serotonergic-dopaminergic mediation of 3,4 methylene-dioxymethamphetamine (MDMA, "ecstasy"). Pharmacol. Biochem. Behav. 31:817–824; 1989.
17. Schechter, M. D.; Meehan, S. M. Ethanol discrimination in Fawn-hooded rats is compromised when compared with other strains. Alcohol 10:77–81; 1993.
18. Signs, S. A.; Schechter, M. D. The role of dopamine and serotonin receptors in the mediation of the ethanol interoceptive cue. Pharmacol. Biochem. Behav. 30:55–64; 1988.
19. Stolerman, I. P.; Shine, P. J.; Samele, C.; Rasul, F. The drug discrimination database, 1951–1988. Rockville, MD: NIDA Administrative Document; 1989.
20. Tallarida, R. J.; Murray, R. B. Manual of pharmacologic calculations with computer program. New York: Springer-Verlag; 1981.
21. Wong, D. T.; Lumeng, L.; Threlkeld, P. G.; Reid, L. R.; Li, T.-K. Serotonergic and adrenergic receptors in alcohol-preferring and nonpreferring rats. J. Neural Transm. 71:207–218; 1988.
22. Wong, D. T.; Reid, L. R.; Li, T-.K.; Lumeng, L. Greater abundance of serotonin$_{1A}$ receptor in some areas of alcohol-preferring (P) rats compared to nonpreferring (NP) rats. Pharmacol. Biochem. Behav. 46:173–177; 1993.