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BRIEF COMMUNICATION

Tolerance and Cross-Tolerance to the Effects of Amphetamine, Methamphetamine and Fenfluramine on Milk Consumption in the Rat[1,2]

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KANDEL, D., D. DOYLE AND M. W. FISCHMAN. *Tolerance and cross-tolerance to the effects of amphetamine, methamphetamine and fenfluramine on milk consumption in the rat.* PHARMAC. BIOCHEM. BEHAV. 3(4) 705–707, 1975. – It has been reported that rats develop tolerance to the milk intake suppressant effects of d-amphetamine, d-methamphetamine, and d, l-fenfluramine. However, it has been hypothesized that the mechanism of action of fenfluramine is different from that of the other two drugs. In the present experiment, rats were given one of these 3 phenylethylamine derivatives each day until tolerance developed to the suppression of milk intake. A second of these drugs was then substituted and milk intake measured. There was cross-tolerance to the drug-induced suppression between d-amphetamine and d-methamphetamine, but not between d-amphetamine and d,l-fenfluramine. The lack of cross tolerance suggests a different mechanism of action for these drugs.

Amphetamine Methamphetamine Fenfluramine Tolerance Cross-tolerance

D-AMPHETAMINE, d-methamphetamine and d,l-fenfluramine are three related phenylethylamine derivatives with similar food intake suppressant effects [1, 3, 6, 8, 9, 10]. Despite this similarity in action, their behavioral effects are not totally comparable. For example, d,l-fenfluramine causes a decrease in spontaneous motor activity while d-amphetamine and d-methamphetamine cause an increase in spontaneous motor activity [2, 3, 6, 8, 9, 10, 11]. It is therefore possible that these drugs, which all cause a decrease in food consumption when administered acutely, are modifying food intake via different mechanisms of action. If so, then tolerance to the appetite-suppressant effects of one of these phenylethylamines might not be expected to persist when another is substituted in the tolerant animal. This research was designed to examine the development of tolerance to the milk intake suppressant effects of d-amphetamine, d-methamphetamine and d,l-fenfluramine in the rat. When tolerance, defined as a return to stable milk intake, developed, a second of these drugs was substituted and intake measured. It was found that initial tolerance developed in all animals for all three drugs. Cross tolerance was demonstrated between d-amphetamine and d-methamphetamine, but not between d-amphetamine and d,l-fenfluramine.

METHOD

The animals were 37 male Holtzman rats, weighing between 340 and 390 g at the beginning of the experiment. Each rat was housed individually and maintained on a feeding schedule of 15 min daily access to 40 ml Bordons Sweetened Condensed Milk (2 parts water: 1 part milk) in the home cage. Water was available at all times except during the 15 min period of access to milk. After the 15 min had elapsed, each rat was given 4–6 g Purina Lab Chow.

After milk intake stabilized for each rat (less than 10 percent variation in intake for 3–6 days), the animals were divided into 9 groups on the basis of matched weight and were given daily subcutaneous injections of 0.1 ml saline 15

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TABLE 1

DRUG TREATMENT SCHEDULE: ORDER, NUMBER OF SUBJECTS IN GROUP, DRUG, DOSE, AND NUMBER OF DAYS ON DRUG REGIMEN

		Drug Regimen No. 1			Drug Regimen No. 2		
Group	N	Drug	Dosage	Days	Drug	Dosage	Days
A–F	4	d-amphetamine	1.5 mg/kg	19	d,l-fenfluramine	10.0 mg/kg	12
A–S	4	d-amphetamine	1.5 mg/kg	19	saline	0.1 ml	12
S–F	4	saline	0.1 ml	19	d,l-fenfluramine	10.0 mg/kg	12
F–A	4	d,l-fenfluramine	10.0 mg/kg	19	d-amphetamine	1.5 mg/kg	12
F–S	4	d,l-fenfluramine	10.0 mg/kg	19	saline	0.1 ml	12
S–A	4	saline	0.1 ml	19	d-amphetamine	1.5 mg/kg	12
A–M	5	d-amphetamine	1.5 mg/kg	19	d-methamphetamine	1.5 mg/kg	12
S–M	4	saline	0.1 ml	19	d-methamphetamine	1.5 mg/kg	12
M–A	4	d-methamphetamine	1.5 mg/kg	26	d-amphetamine	1.5 mg/kg	5

min prior to each milk feeding. When milk intake was again stable, the first daily drug regimen was begun. Table 1 presents the order of drug administration and number of animals in each of the 9 groups. The doses selected produced equal effects on milk intake when administered acutely during a pilot study prior to this experiment. All drug doses, given daily in subcutaneous injections 15 min prior to each milk-feeding period, have been shown to be nonlethal in the rat and produced food intake suppression to which tolerance develops [1, 2, 3, 4, 6, 9, 10]. The drugs were in the salt from and dissolved in 0.1 ml of 0.9 percent saline for injection.

During the 19 days (26 days for group M-A, because tolerance developed more slowly) of the first drug regimen, tolerance to the drugs' suppressant effect on milk intake developed for all rats. This was defined as a return of milk intake to the initial saline baseline or a new stable baseline. Rats given saline maintained stable milk intake. The second drug regimen was then instituted for 12 days (5 days for Group M-A), a period of time sufficiently long for milk intake to approach stable baseline levels.

RESULTS

No cross-tolerance to the milk intake suppressant effects of d-amphetamine and d,l-fenfluramine was demonstrated in this study. In Group A-F, tolerance developed to the suppressant effects of d-amphetamine, but when, d,l-fenfluramine was substituted, milk consumption decreased. Similarily, in Group F-A, tolerance developed to the suppressant effects of d,l-fenfluramine but d-amphetamine again caused a decrease in milk consumption. A comparison of milk intake during the last 5 days on the initial drug regimen to intake during the 5 consecutive days of maximal decrease in milk consumption on the second drug regimen for both Groups A-F and F-A shows the difference to be significant (t, two-tailed = 4.33, 3.68 $p<0.25$).

A closer analysis of the data reveals that neither the decrease in milk consumption nor the pattern of tolerance development on the second drug regimen was affected by the initial drug administration. This is shown by the remarkable similarity of the effects of d,l-fenfluramine in Groups A-F and S-F (Fig. 1A) and of d-amphetamine in Groups F-A and S-A (Fig. 1B). In addition, groups A-S (Fig. 1A) and F-S (Fig. 1B) demonstrate that it was possible to recover initial stable milk intake baselines when saline was substituted for drug.

There is cross-tolerance to the milk intake suppressant effects of d-amphetamine and d-methamphetamine. This can be seen by comparing the data from Group A-M (Fig. 1C) and Group M-A (Fig. 1D). Tolerance developed to the suppressant effects of the initial drug, d-amphetamine. Upon substitution of d-methamphetamine, no decrease (and even a slight increase) in milk consumption occurred. In addition, the order of drug presentation is unimportant to cross-tolerance development as shown in Group M-A. With substitution of d-amphetamine for d-methamphetamine, no decrease in milk consumption occurred. The presence of cross-tolerance may be more clearly shown by comparison of the data from Group A-M and M-A with the data from Groups S-M and S-A. Rats given d-methamphetamine and d-amphetamine in Groups S-M and S-A, respectively, showed significant (t, two-tailed = 2.74, 5.03 $p<0.05$) decreases in milk consumption while rats given d-methamphetamine and d-amphetamine in Groups A-M and M-A, respectively, showed no decrease. The tolerance to d-methamphetamine in Group A-M is contingent on prior administration of d-amphetamine, just as the tolerance in

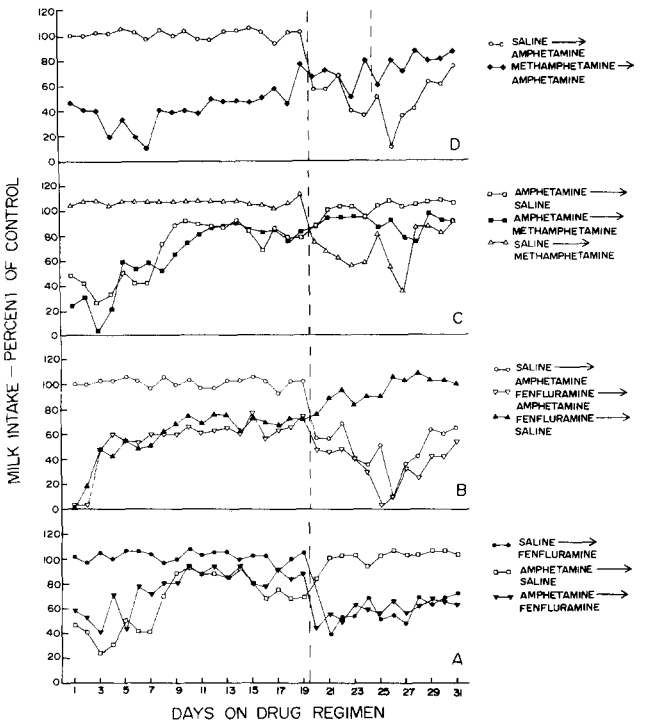

FIG. 1. Effects of d-amphetamine, d-methamphetamine and d,l-fenfluramine on milk consumption in rats. Points are expressed as a percent of the pretreatment saline level of each group. The vertical dashed lines indicate the day chronic drug regimen No. 2 was substituted for No. 1 drug regimen. For the methamphetamine-amphetamine group this was Day 26; for the other groups it was Day 19.

Group M-A is contingent on prior administration of d-methamphetamine.

The patterns of milk intake changed differentially after the daily administration of d-amphetamine and d,l-fenfluramine. Milk intake after d-amphetamine showed maximal decreases after a few days of daily drug administration. On the other hand, milk intake decreased maximally on the first day of daily administration of d,l-fenfluramine. D-methamphetamine was quite similar in effect to d-amphetamine except that the development of tolerance was more variable than either d-amphetamine or d,l-fenfluamine. The differences in the patterns of milk intake between the points of maximal disruption, however, may be due to the differences in cumulative effects of these drugs.

DISCUSSION

The results of this study demonstrate the lack of cross-tolerance to the milk intake suppressant effects of d-amphetamine and d,l-fenfluramine. Rats tolerant to the milk intake suppressant effects of d,l-fenfluramine and rats maintained on a saline regimen both showed similar decreases in milk consumption when given d-amphetamine. Further, the lack of cross-tolerance was found to be independent of the drug order. Rats tolerant to d-amphetamine and rats maintained on a saline regimen both showed similar decreases in milk consumption when given d,l-fenfluramine.

Cross-tolerance did occur, however, between d-amphetamine and d-methamphetamine. Rats tolerant to the effects of d-methamphetamine were found to be tolerant to the effects of d-amphetamine. Again, results were independent of the drug order.

Recent studies have indicated that d,l-fenfluramine and d-amphetamine act by different mechanisms and possibly at different sites [5,7]. These studies suggested that fenfluramine milk intake suppression involved serotonin mechanisms whereas amphetamine milk intake suppression involved catecholamine mechanisms. Our study supports the hypothesis that these drugs may, in fact, have different mechanisms of action, in that both the lack of cross-tolerance and the differing patterns of tolerance development indicate that amphetamine and fenfluramine produce milk intake suppression via different mechanisms. The differential effects of these drugs on the CNS (d-methamphetamine and d-amphetamine increase spontaneous motor activity while, d,l-fenfluramine decreases it) lend further credence to this concept of different mechanisms of action. The results of this research demonstrate that the use of a tolerance-cross tolerance design can be an important tool in studying some of the relationships between pharmacological compounds and behavior.

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