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Effect of Dopamine Agonists and Fenfluramine on Discriminative Behavior in Obese and Lean Zucker Rats

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SCHECHTER, M. D. AND J. A. FINKELSTEIN. *Effect of dopamine agonists and fenfluramine on discriminative behavior in obese and lean Zucker rats.* PHARMACOL BIOCHEM BEHAV **23**(1) 7–11, 1985.—Dopamine agonists and fenfluramine were used as pharmacological probes to investigate the possible difference in sensitivity and time course of drug action in genetically obese Zucker rats and their lean littermates. All rats were trained to discriminate between the stimulus properties of 0.6 mg/kg *d*-amphetamine and its vehicle in a two-lever, food-motivated operant task. Once trained, both groups of rats showed a dose-related decrease in discriminative performance with lower amphetamine doses. Analysis of the dose-response curves indicated an ED50 for the obese rats of 0.17 mg/kg and for the lean group of 0.14 mg/kg. Administration of 0.3–1.2 mg/kg *l*-amphetamine and 2.5–10.0 mg/kg cocaine produced a pattern of responding similar to that observed with *d*-amphetamine. In contrast, 0.08–mg/kg apomorphine produced saline-appropriate responding and 1.5–2.5 mg/kg fenfluramine produced intermediate results in both groups. Time-course experiments indicated that the lean rats maintain errorless discriminative performance through 90 min post-injection, whereas the obese rats discriminate *d*-amphetamine significantly less at that post-administration time. The results suggest a similar sensitivity to *d*-amphetamine and other dopaminergic agonists in obese and lean rats with a difference in the time-course of *d*-amphetamine's action between these two groups.

Drug discrimination Amphetamine Fenfluramine Zucker rats Dopamine Stimulus properties of drugs

AMPHETAMINE and fenfluramine possess similar chemical structures and are both clinically effective appetite suppressants. However, they appear to differ in their effects upon eating behavior, psychomotor performance, and brain neurotransmitters. Thus, amphetamine has been reported to delay the onset of eating in rats, whereas fenfluramine allowed eating to commence normally but brought about early termination suggesting that amphetamine suppresses hunger and fenfluramine promotes satiety [1]. Amphetamine potentiates central catecholamine transmission to increase behavioral responding [12], whereas fenfluramine appears to increase brain serotonergic activity and inhibit behavioral responding [4]. These studies were generally performed in normal Sprague-Dawley or Wistar rats; the study of the effects of anorexiants in an obese animal model would better simulate conditions for their therapeutic use.

Although the genetically obese rat from the Zucker strain [20] is generally regarded as one of the most appropriate models of juvenile onset human obesity [17], little is known regarding its response to the commonly used anorectic drugs. Results of one study [10] indicated that obese Zucker rats were as responsive to low doses of amphetamine as lean rats, whereas another study [6] showed that the obese rat was more susceptible to the anorexic action of *d*-amphetamine but equally affected by fenfluramine.

The drug discrimination procedure is a sensitive, specific and reliable technique in which trained laboratory animals can indicate whether or not they have received administration of a specific drug. Typically, food-deprived rats are trained to press one lever to receive food when a drug has been administered or to press an alternate lever to receive food when saline has been administered. When discrimination between the drug and non-drug (saline) state is learned and retained, tests with various doses of the trained drug can indicate the animals' behavioral sensitivity to the drug. In addition, tests with other drugs can be conducted and this testing can provide information concerning the similarities of stimulus properties of other drugs to that of the trained drug. Both *d*-amphetamine [7, 14] and fenfluramine [5, 9, 19] have been reported to act as drugs capable of controlling discriminative responding in normal rats. The purpose of the present study was to train obese Zucker rats and their lean littermates to discriminate the interoceptive cue produced by *d*-amphetamine and to test the ability of other dopaminergic agonists, as well as another class of anorexiant, i.e., fenfluramine, to substitute for the trained drug condition. The ultimate objective, therefore, was to use these agents as pharmacological probes to investigate possible differences in drug sensitivity and time-course of drug action in normal and genetically obese rats.

METHOD

Subjects

Four genetically obese (fa/fa) and four lean (Fa/−) female mature Zucker rats were obtained from the breeding colony

TABLE 1

DOSE-RESPONSE GENERALIZATION WITH AMPHETAMINE, OTHER DOPAMINERGIC AGONISTS AND FENFLURAMINE

Treatment	Dose (mg/kg)	No. Trials	Lean		Obese	
			Quantal	Quantitative (SD)	Quantal	Quantitative (SD)
Saline	—	28	0.0	17.4 (8.4)	0.0	8.6 (5.1)
d-Amphetamine	0.6	28	96.9	81.1 (10.4)	96.9	89.5 (8.5)
	0.3	2	62.5	57.6 (6.9)	87.5	78.5 (2.2)
	0.15	2	62.5	61.9 (12.9)	50.0	48.0 (27.6)
	0.08	2	25.0	34.0 (2.8)	0.0	4.6 (6.4)
l-Amphetamine	1.2	2	75.0	71.2 (5.2)	100.0	87.1 (18.3)
	0.6	2	62.5	57.5 (9.0)	50.0	40.5 (1.9)
	0.3	2	25.0	37.0 (25.8)	0.0	7.7 (10.0)
Apomorphine	0.24	2	25.0	41.0 (0.6)	12.5	14.9 (14.3)
	0.16	2	0.0	24.9 (16.8)	0.0	4.8 (0.6)
	0.08	2	0.0	17.3 (6.3)	0.0	4.8 (0.0)
Cocaine	10.0	2	100.0	88.5 (0.0)	87.5	80.3 (11.7)
	5.0	2	75.0	68.9 (23.6)	62.5	60.2 (10.0)
	2.5	2	12.5	36.6 (5.9)	12.5	24.6 (11.2)
Fenfluramine	2.5	2	62.5	54.9 (16.0)	50.0	52.0 (10.8)
	2.0	2	12.5	31.3 (6.0)	12.5	25.8 (8.2)
	1.5	2	0.0	12.1 (6.7)	0.0	1.5 (2.1)

at the Northeastern Ohio Universities College of Medicine. Obese rats weighed a mean of 630.2 g (range 497–702 g) and their lean littermates weighed a mean of 317.0 g (range 302–361 g). They were housed in individual cages and their weights were adjusted, by daily rationing of commercial rat chow, to approximately 80–85% of their initial weights. Water was continuously available in the home cages which were kept at a constant temperature (20–22°C) and maintained on a 12-hour light/12-hour dark daily cycle.

Apparatus

The experimental space consisted of four identical standard rodent operant chambers (Lafayette Instruments Corp., Lafayette, IN) each equipped with two levers located 7 cm apart and 7 cm above the grid floor. A food pellet receptacle was mounted 2 cm above the grid floor at an equal distance between the two levers. The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and a 9W house-light. Solid-state programming equipment (LVB Corp., Lehigh Valley, PA) was used to control and record the sessions and was located in an adjacent room.

Discrimination Training

Drug discrimination training was based upon procedures described in detail elsewhere [16]. There were two training phases. In the first phase, the food-deprived Zucker and lean rats learned to press the lever indicating saline administration and received a food reward (45 mg Noyes pellet) for each correct response, fixed ratio 1 (FR1) schedule. This schedule was made progressively more difficult, in daily 15 min sessions, over 10 days until an FR10 schedule was achieved. Throughout lever press training, all rats received daily intraperitoneal (IP) injections of saline (0.9% sodium chloride) 20 min prior to being placed into the two-lever operant box. Immediately following the attainment of the FR10 schedule after saline administration, the opposite lever was activated and rats received a food reward for each correct response, fixed ratio 1 (FR1) schedule, after the IP administration of an equal volume (1 ml/kg body weight) of saline containing 0.6 mg/kg *d*-amphetamine sulfate (as base). Daily sessions, of 15 min duration, were continued over 8 days with drug administration until an FR10 schedule was attained. In order to minimize effects due to any possible position preference, the rats in each group were divided into two subgroups. For one subgroup (n=2) responding on the left lever was reinforced by delivery of food pellets in every session following drug injection, whereas the other group (n=2) was reinforced with food after responding on the right lever following drug injection. Responses on the opposite lever were reinforced with food pellets after saline injection.

Phase II discrimination training then began. Subjects were trained 5 days per week with reinforcement in a pseudo-random sequence. Thus, in each two week period, there were five days with drug lever (D) and five days with saline lever (S) correct. The pattern was D, S, S, D, D; S, D, D, S. S. The training criterion was reached when the animal selected the appropriate lever, according to the drug state imposed, on eight of ten consecutive sessions.

Dose-Response Relationships

After both groups of rats attained the discriminative training criterion, testing and training sessions of 15 min duration with alternating administrations of either 0.6 mg/kg

TABLE 2

TIME-COURSE OF D-AMPHETAMINE IN LEAN AND OBESE ZUCKER RATS

Condition	Time (min)	No. Trials	Lean Quantal	Lean Quantitative (SD)	Obese Quantal	Obese Quantitative (SD)	p*
Saline	20	12	0.0	9.1 (2.1)	0.0	6.0 (1.9)	NS
Amphetamine	10	2	87.5	82.9 (11.4)	100.0	95.2 (0.0)	NS
0.6 mg/kg	20	12	100.0	92.9 (2.8)	100.0	95.1 (2.7)	NS
	60	2	100.0	90.6 (2.6)	75.0	74.5 (2.6)	0.01
	90	2	100.0	87.5 (8.0)	62.5	57.9 (10.4)	0.04
	180	2	62.5	52.2 (10.5)	12.5	17.1 (5.8)	0.03
	240	2	25.0	35.2 (0.0)	0.0	13.4 (2.7)	0.004

*Level of significance between quantitative measurements of lean and obese rats at each post-injection time (Student t-test of means).

d-amphetamine or saline were continued on Mondays, Wednesdays, and Fridays. It was intended that if a rat was observed to make more than two incorrect lever selections in any of 10 consecutive maintenance sessions, the data on that rat's performance would be deleted from the results. This, however, did not occur. On Tuesdays and Thursdays, the rats of each group were injected IP with one of several different doses of d-amphetamine than used for initial training and 20 min later, they were placed into the experimental chamber. They were allowed to lever press, without receiving reinforcement, until ten presses were made on either lever. To preclude training at a drug dose different than employed to train the animals, the rats were immediately removed from the experimental chamber once the total responses on one lever reached 10 presses. Each of the test doses of drug was tested in each animal on two occasions with each test preceded both by a drug and a saline maintenance session. The lever first pressed ten times was designated as the "selected" lever and the percentage of rats choosing the drug-correct lever constitutes the quantal measurement (below).

Time-Course of d-*Amphetamine*

Following the dose-response experiments, testing days were used to test the time-course of action of the training dose of d-amphetamine. Thus, each group of rats was administered 0.6 mg/kg d-amphetamine IP and they were tested at various times after drug administration. All post-injection times were randomized and each time was preceded by both a saline and d-amphetamine maintenance session at 20 min post-injection. Post-injection times of 10, 60, 90, 180, and 240 min were employed and at the end of each of these times, the rats were allowed to choose one of the two levers without reinforcement and once either lever was pressed 10 times, the rat was immediately removed to preclude further training.

Substitution Tests

Subsequently, testing days (Tuesdays and Thursdays) were used to investigate the ability of the amphetamine-trained rats to discriminate numerous drugs evidenced to act upon dopaminergic neurons, viz., l-amphetamine, cocaine, and apomorphine, as well as fenfluramine, at doses reported in the literature to produce behavioral effects.

Measurements and Statistical Treatment

The lever pressed 10 times first was designated as the "selected" lever. The percentage of rats selecting the lever appropriate for the training drug was the quantal measurement of discrimination. In addition, the total number of lever presses on both levers made before ten presses on either lever were counted constitutes the quantitative measurement, i.e., the number of responses on the drug-correct lever divided by total responses made prior to ten responses, times 100. The advantages in using both measurements have been discussed by Stolerman and D'Mello [18]. The quantal data for the dose-response experiments were analyzed by the method of Litchfield and Wilcoxon [8] which employs probit vs. log-dose effects and generates ED50's and tests for parallelism. The quantitative data were analyzed by a Student's t-test of means with $p<0.05$ selected as the criterion for significant differences.

RESULTS

Acquisition of Discrimination

The four obese rats required a mean (±SD) of 1.75 (0.5) training sessions (range 1–3) to attain discriminative criteria, i.e., to reach the first of ten consecutive sessions in which 8 of 10 lever selections were correct according to the drug state imposed. Likewise, the lean rats rapidly learned to discriminate between 0.6 mg/kg d-amphetamine and saline with a mean of 2.75 (1.5) sessions (range 2–5).

Dose-Response Relationship With d-*Amphetamine*

The training dose of 0.6 mg/kg d-amphetamine produced 96.9% of first choice responses (selected lever; quantal measurement) upon the drug-appropriate lever in both groups of rats during all maintenance sessions, whereas saline administration produced errorless discrimination, i.e., during no trial was the drug-correct lever chosen after saline administration (Table 1). Decreasing doses of d-amphetamine generally produced decreased discriminative performance in all subjects. Analysis of the dose-response curves [8] indicated an ED50 of 0.143 (95% confidence range:

0.071–0.288) mg/kg for the lean rats and an ED50=0.170 (0.0958–0.305) mg/kg for the obese rats. Test for parallelism of the two dose-response curves [8] indicated that they were parallel, i.e., critical $t=2.776$ > calculated $t=0.550$.

Generalization to Other Dopaminergic Agonists

Administration of *l*-amphetamine and cocaine produced a pattern of discriminative responding similar to *d*-amphetamine in both groups of rats. The ED50 for *l*-amphetamine in lean rats was 0.536 mg/kg and in obese rats 0.596 mg/kg; dose response lines being parallel (critical $t=4.303$ > calculated $t=1.713$). Likewise, cocaine produced dose-responsive discriminative generalization with the ED50 in the lean group being 4.169 mg/kg and in the obese rats, 4.632 mg/kg. In contrast, all of the three doses of apomorphine produced saline-appropriate responding in both groups.

Generalization to Fenfluramine

The highest dose of (2.5 mg/kg) fenfluramine produced only partial transfer of lever selection in the *d*-amphetamine-trained rats. A higher fenfluramine dose was precluded by indications of behavioral disruption.

Time-Course of d-*Amphetamine Action*

Maintenance sessions, with both saline and 0.6 mg/kg *d*-amphetamine at 20 min post-administration, produced errorless discriminative performance (Table 2). This 100% discrimination continued in the lean rats through 90 min post-injection with a decline at 180 and 240 min. In contrast, the obese rats discriminated *d*-amphetamine significantly poorer than the lean rats (quantitative measurement; Table 2) at 60 min and at all later post-injection times.

DISCUSSION

The results of the present study indicate that *d*-amphetamine can serve as a discriminative stimulus both in normal and genetically obese rats and decreasing doses of the drug generates a standard dose-response curve. The *d*-amphetamine ED50's, for the obese and lean rats of 0.170 and 0.143 mg/kg, respectively, indicates that the two groups of rats were similarly responsive to the discriminative properties of this drug. This extends a previous report in which obese and lean Zucker rats were shown to be equally sensitive to the anorexic effects of 0.5 and 1.0 mg/kg *d*-amphetamine [10].

Administration of various doses of the l-isomer of amphetamine produced a dose-responsive transfer of discrimination in both obese and lean rats with the ED50 in the former being 0.596 mg/kg and in the latter, 0.536 mg/kg. The d-isomer was thus, 3.5 to 3.7 times as potent as the l-isomer and the dose-response curves were parallel within statistical limits. This approximates the l:d potency ratio of 4.9 previously reported [13] and is consistent with the concept that the two isomers act by the same mechanism, presumably via dopaminergic mediation [14]. Likewise, administration of numerous doses of cocaine were observed to produce discriminative responding similar to that of *d*-amphetamine as previously reported [2,3]. In contrast, apomorphine administration produced saline-appropriate responses.

Fenfluramine, at the doses tested, produced only partial transfer of amphetamine discrimination. Previous work indicated that fenfluramine did not transfer in amphetamine-trained rats [15] and that amphetamine did not generalize in fenfluramine-trained rats [5, 9, 19]. Thus, the differences in the discriminative stimulus functions of *d*-amphetamine and fenfluramine have been suggested to be mediated by dopaminergic [14] and serotonergic [9] neuron systems, respectively. Indeed, fenfluramine has recently been reported to have no effect upon the brain level or metabolism of dopamine in obese or lean rats [11].

Although the sensitivity of these obese and lean rats to the various dopamine agonists and fenfluramine were similar, a significant difference in discriminative performance between these two groups was observed in a time-course study utilizing the training dose of *d*-amphetamine. The lean rats were observed to discriminate amphetamine errorlessly for 90 min post-administration, whereas the obese rats were observed to discriminate at this level only for 20 min. At 60, 90, 180, and 240 min post-injection, there was a significant decrease in the quantitative performance of the obese rats when compared to their lean littermates. The most parsimonious explanation for this result resides in the possibility that the lipid-soluble *d*-amphetamine was removed more quickly from the blood stream and sequestered in the more abundant fat tissue of the obese rats, thus decreasing brain concentrations of the drug in time. It must be kept in mind that, in these experiments, obese and lean rats were injected with the same dose/kg body weight. As a result, the obese rats received approximately twice the absolute dose as the lean; however, their metabolic mass is also approximately twice as great and it would be expected that drug actions would be affected by metabolic mass [10]. Thus, any inference regarding the site of action or potency of these drugs must be qualified by recognition of the drugs' distribution to specific tissue sites.

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