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BRIEF COMMUNICATION

Lack of Generalization of Nisoxetine With Amphetamine in the Rat

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SCHECHTER, M. D. AND J. W. BOJA. *Lack of generalization of nisoxetine with amphetamine in the rat.* PHARMACOL BIOCHEM BEHAV **30**(4) 1085–1088, 1988.—Rats were trained to discriminate between the stimulus properties of intraperitoneally administered *d*-amphetamine (0.8 mg/kg) and its vehicle in a two-lever, food-motivated operant task. Once trained, doses of the norepinephrine reuptake inhibiting agent nisoxetine, ranging from 10 to 20 mg/kg, were administered to investigate if the amphetamine-trained rats would generalize to this agent. This did not, however, occur. Thus, it would seem that noradrenergic mechanisms have a negligible role in the production of the amphetamine-induced discriminative stimulus cue in the rat. Previous evidence that indicated a noradrenergic mediation of amphetamine discrimination in the mouse contrasted with the present results in rats and this discrepancy should warrant caution in comparing results of discriminative studies in these two species.

Amphetamine Drug discrimination Dopamine Nisoxetine Norepinephrine Rat

THE behavioral paradigm employing the discriminative stimulus properties of drugs has proven to be an important and well-evidenced tool in behavioral pharmacology. The psychostimulant and anorexiant drug amphetamine has been shown to serve as an effective discriminative stimulus in many animal species and it is perhaps one of the most popular and well-researched of all the drugs used to train animals in this behavioral paradigm [10]. In investigations that sought to determine the mechanism of action of amphetamine, a large body of evidence would suggest that amphetamine produces its discriminative effects via dopaminergic neurons (e.g., [3,10]).

The outstanding exception to this evidence was a study involving the initial use of mice in a drug discrimination paradigm [4]. In a task employing a nose-poke response into opposite sides of a corridor within a chamber, mice were trained to discriminate 1.0 mg/kg amphetamine from saline. This discrimination was subsequently shown to generalize to nisoxetine (32 mg/kg). In addition, mice trained to this dose of nisoxetine generalized to 0.56, 1.0 and 3.0 mg/kg amphetamine. Since nisoxetine is a potent inhibitor of brain norepinephrine reuptake, without a similar effect on dopamine [7,8], these results are consistent with the notion that the discriminative stimulus properties of amphetamine in the mouse are mediated by noradrenergic neurons. To date, nisoxetine has not been investigated in the more commonly used species of rat. The purpose of the present study was to do that and, in light of the suggestion that the mouse be used more often in drug discrimination studies [1], to determine if the species difference in these two rodents would explain this and other possible (forthcoming) discrepancies.

METHOD

Subjects

Eight male Sprague-Dawley rats (Zivic-Miller Laboratories, Allison Park, PA) were the subjects used in these experiments. They were individually housed in a room on a 12-hr (0600–1800) light/12-hr dark schedule and at a constant temperature (22°C) and humidity (40–42%). Tap water was available in the home cage ad lib and rat weights were adjusted to approximately 80–90% of free-feeding weights by daily rationing of commercially-available rat chow. This provided motivation to respond in the food rewarded task.

Apparatus

The experimental equipment consisted of 8 identical standard rodent operant chambers (Lafayette Instrument Corp., Lafayette, IN) each equipped with 2 operant levers located 7 cm apart and 7 cm above the grid floor. A food pellet receptacle was mounted 2 cm above the floor at an equal distance between the levers. The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and a 9-W houselight. Solid-state programming equipment

(Med Associates, E. Fairfield, VT) was used to control and record the sessions and was located in an adjacent room.

Lever-Pressing and Drug Discrimination Training

The drug discrimination consisted of training rats to press one of two available levers in an operant chamber while the rat was under the influence of the drug state (amphetamine) and to press the opposite lever in the nondrug state (vehicle; distilled water). Thus, each of the two stimuli was associated with responding on a particular lever. Initially, lever pressing for food reward was trained (shaped) by placing the food-deprived rat into the operant chamber and delivering a food pellet whenever the exploratory nature of the rat brought it into close proximity of the assigned training lever. The rat soon learned to press the lever for reinforcements (45 mg Noyes food pellets) on a gradually increasing (1–10) fixed ratio (FR) schedule. Rats were initially administered an intraperitoneal (IP) injection of 1 ml/kg of vehicle and 20 min later were placed into the operant chamber. Upon pressing the desired designated correct lever, they received food reinforcement on an FR 1 schedule, i.e., each press resulted in the delivery of a food pellet. The food reinforcement schedule was gradually increased over 10 sessions until the rat was pressing the vehicle-appropriate lever on an FR 10 schedule, i.e., 10 lever presses were required for each food pellet reinforcement.

Subsequently, the rats were administered an equal volume (1 ml/kg) of vehicle containing 0.8 mg/ml of *d*-amphetamine sulfate (calculated as the salt). At 20 min postadministration, they were required to press the opposite lever (to that which they had learned to press after vehicle injection) on an FR 1 schedule in order to receive reinforcement. The training continued, in daily 15 min sessions, proceeding from FR 1 through FR 10, until the amphetamine-appropriate lever was consistently pressed on an FR 10 schedule. In order to minimize the effects due to any possible position preference, the rats were randomly divided into two equally-sized subgroups at the beginning of the experiment. For one subgroup, responding on the left lever following amphetamine administration was reinforced by delivery of food pellets, whereas the other subgroup was reinforced with food after responding on the right lever. The responses on the opposite lever in each case were reinforced with food after vehicle administration.

After the rats were consistently pressing both levers on the FR 10 schedule, discriminative training began utilizing a pseudo-random biweekly schedule of amphetamine (A) or vehicle (V) administration in the following order: A-V-V-A-A; V-A-A-V-V. Thus, in each two week-period, the rats received 5 amphetamine and 5 vehicle administrations. The number of responses on each lever before obtaining the first food pellet was recorded and the first lever pressed ten times was designated as the "selected" lever. The rats were then allowed to continue lever pressing until 400 responses were made on the correct lever and, thus, 40 food reinforcements (on the FR 10 schedule) were obtained. The rats were required to remain on this traning schedule until each rat was able to reach criterion performance. This criterion was met when the rat selected the appropriate lever (according to the drug or nondrug state imposed) correctly in 8 of 10 consecutive daily sessions, twice.

Dose-Response Testing

Once training criterion was achieved by all rats, they were tested with doses of amphetamine that were different than that (0.8 mg/kg) used in their training; this allowed for a dose-response relationship to be observed. During this series of experiments, the maintenance of the amphetamine-vehicle discrimination was assured by administering and testing either 0.8 mg/kg amphetamine or vehicle on every second day. The other doses of amphetamine were tested on interspersed days according to the following schedule: A-DR_1-V-DR_2-A-DR_2-V-DR_1, etc., where A=0.8 mg/kg *d*-amphetamine, V=vehicle, DR_1=one dose of amphetamine, and DR_2=second dose of amphetamine. Employing the same time delay as used in their training, i.e., at 20 min postinjection, the rats were placed into the experiment chamber and allowed to press without reinforcement until 10 responses were made on either of the two levers. When these 10 responses were made the animal was immediately removed from the chamber to preclude reinforcement and/or training at an amphetamine dose other than that to which they were trained. The lever pressed 10 times was designated as the "selected" lever and each amphetamine test dose was administered in a random order on two occasions with test session preceded by one vehicle and one amphetamine test session. In this way the animals' experience on days preceding test days were counterbalanced with respect to any possible aftereffects that may have been produced by the maintenance/training condition.

Nisoxetine Generalization

Once the rats had completed the dose-response experiments, a schedule of substitution testing was begun. In the substitution experiments, various doses (10, 12.5, 15 and 20 mg/kg) of the drug nisoxetine (as the HCl salt) were administered IP 20 min prior to testing on two occasions each in sessions interspersed between maintenance days. The lever pressed 10 times was recorded and the animals were immediately removed, without receiving food reinforcement, upon making this selection.

Measurements and Statistical Analysis

The percentage of rats selecting the lever appropriate for the training drug (amphetamine) was the quantal measurement of discrimination. Quantal data are presented as percentage of rats making correct first-choice selection on the amphetamine-correct lever (all-or-none). The dose-response measurements were subjected to analysis by the procedure of Litchfield and Wilcoxon [2] that employs log dose vs. probit measurements.

The quantitative measurement used represents the total number of lever presses on both levers made before completion of ten presses on either lever, i.e., the number of responses on the amphetamine-correct lever divided by the total responses made (including the ten on the amphetamine lever) times 100. This measurement was included to analyze data on both levers and to be able to incorporate counts upon the "unselected" lever in the statistical analysis.

RESULTS

Maintenance day testing with 0.8 mg/kg *d*-amphetamine resulted in errorless discrimination as did testing with its vehicle (Table 1). Thus, on alternating days amphetamine produced 100% of first choice responses upon the amphetamine-appropriate lever while, on other days, distilled water (vehicle) produced no responding upon this lever

TABLE 1

SUBSTITUTION OF VARIOUS DOSES OF *d*-AMPHETAMINE AND NISOXETINE IN RATS (n=8) TRAINED TO DISCRIMINATE 0.8 mg/kg *d*-AMPHETAMINE

Drug	Dose (mg/kg)	No. Trials	Quantal	Quantitative (SD)
Vehicle	—	12	0.0	10.7 (7.5)
d-Amphetamine	0.8	12	100.0	91.9 (2.7)
	0.4	2	72.2	69.5 (20.8)
	0.2	2	22.2	30.5 (19.4)
Nisoxetine	20.0	2	18.8	29.2 (6.4)
	15.0	2	31.3	34.6 (1.8)
	12.5	2	12.5	30.4 (5.1)
	10.0	2	18.8	29.2 (4.0)

and, therefore, 100% of all selections upon the vehicle-appropriate lever. Decreasing doses of amphetamine produced decreased frequency of drug lever choices both in terms of quantal and quantitative measurements. The ED_{50} for amphetamine was 0.31 mg/kg in probit analysis [2] of the quantal data and a similar 0.28 mg/kg for the quantitative data.

When interspersed test days were employed to investigate the effect of nisoxetine on animals trained to discriminate amphetamine, the resulting discrimination was generally vehicle-like in nature. Doses of nisoxetine from 10–20 mg/kg never produced greater than 31.3% (15 mg/kg) of first choice responding on the amphetamine-correct lever. Both of the higher doses used, in addition, produced behavioral disruption, i.e., delays prior to 10 presses on either lever.

DISCUSSION

The present investigation once again indicates that amphetamine is capable of functioning as drug to control differential discriminative performance in the rat; this has been shown to occur in this [3] and many other [10] laboratories. Likewise, decreasing doses of amphetamine administered to these animals produced decreased discriminative performance and generated a typical dose-response relationship with an ED_{50} of 0.31 mg/kg. Throughout this large body of scientific literature regarding the discriminative stimulus properties of amphetamine is the observation that this stimulus is mediated by dopaminergic postsynaptic neurons in the brain. This notion has been evidenced by the ability of pretreatment with alpha-methylparatyrosine, an inhibitor of catecholamine synthesis, to inhibit the amphetamine discriminative cue, whereas depletion of serotonin by pretreatment with para-chlorophenylalanine had no significant effect [3]. Additional evidence as to the dopaminergic pathway mediation of the discriminable effects of amphetamine resides in the ability of numerous clinically-effective antipsychotic drugs, whose mechanism of action is thought to be by blockade of dopaminergic postsynaptic receptors, to inhibit the amphetamine discriminative cue in many species of animals trained in this paradigm [10]. In contrast, pretreatment with drugs which affect other neuronal systems (e.g., disulfiram, phenoxybenzamine, phentolamine, atropine and propranolol) were without effect [10].

Nisoxetine (dl-N-methyl-3-(o-methoxyphenoxy)-3-phenylpropylamine), aka Lilly 94939, has been shown to be a potent inhibitor of the reuptake of norepinephrine into synaptosomes of the rat brain [8] and this effect was shown to be 200-fold greater than its action upon dopamine reuptake inhibition [7]. Pretreatment with this agent has been reported to abolish the locomotor stimulating effect of amphetamine and to potentiate the latter drugs' actions on stereotypy in mice [6]. Of more importance to the present work, nisoxetine was reported to share common discriminative stimulus properties as amphetamine in mice trained to discriminate either of the two agents [4]. This was later confirmed by the same laboratory [5]. The present study, using the rat as the subject to discriminate amphetamine, would indicate that nisoxetine does not have a similar effect in this species. Thus, no dose of nisoxetine from 10–20 mg/kg produced amphetamine-like responses in animals trained to discriminate 0.8 mg/kg *d*-amphetamine from its vehicle. In lieu of biochemical analysis, the possibility exists that nisoxetine may not have allowed for the accumulation of enough norepinephrine to produce this generalization in the rat.

In conclusion, this work sought to replicate the generalization of nisoxetine in amphetamine-trained animals using rats in contrast to mice and found that there may, indeed, be a species difference between these two rodents concerning the mechanism by which amphetamine serves as a discriminative cue. Furthermore, a recent report indicates that rhesus monkeys trained to discriminate the direct dopamine agonist apomorphine do not generalize to nisoxetine [9]. Therefore, mice, which have rarely been used for drug discrimination research, must be viewed with caution as to the central mediation of discriminative effects when compared to the more universally employed rat.

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