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Functional Consequences of Fenfluramine Neurotoxicity

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SCHECHTER, M. D. *Functional consequences of fenfluramine neurotoxicity*. PHARMACOL BIOCHEM BEHAV **37**(4) 623–626, 1990.—Male Sprague-Dawley rats were trained to discriminate the anorectic drug d,l-fenfluramine (2.0 mg/kg intraperitoneally administered) from its vehicle using a food-motivated (fixed-ratio 10 schedule) two-lever operant task. Once trained, doses of 0.5, 1.0 and 1.5 mg/kg fenfluramine tested 20 min after IP administration produced dose-responsive discrimination performance. Subsequently, noncontingent twice-a-day administrations of 1 ml/kg saline were made for 4 days and the dose-effect relationship redetermined on the 13th to 15th day after initiation of the chronic saline regimen. Results of these dose-response experiments indicated that there was no significant effect upon fenfluramine discrimination after multiple saline injections or after 10 days without training. Following four days of retraining, 6.25 mg/kg fenfluramine twice-a-day for four days was followed 10 days later by another dose-response determination. This purportedly neurotoxic regimen of fenfluramine significantly increased the rats' ability to discriminate fenfluramine. These results suggest the possibility that chronic release of serotonin or selective damage to serotonin-containing neurons produced by fenfluramine may lead to postsynaptic supersensitivity as manifested by the functionally increased discriminative performance observed.

Fenfluramine Serotonin Stimulus properties of drugs Drug discrimination Supersensitivity Rats

FENFLURAMINE is an m-trifluoromethyl-N-ethyl derivative of amphetamine which has been used for more than 25 years for the treatment of obesity (19) and, more recently, has been shown to decrease autistic symptomatology in approximately one-fourth of the children who have received daily doses of it for several months (2, 13, 18). The anorectic effects of fenfluramine are believed to be due to its serotonin (5HT)-releasing properties (5, 8, 20), as well as its ability to inhibit serotonin reuptake (6,7), two mechanisms expected to increase synaptic serotonin concentrations and, thereby, to hyperstimulate postsynaptic 5HT neurons. A recent research news article, appearing in *Science* (3), likened the continued use of fenfluramine in obesity control, and the possible 5HT neurotoxicity that it may produce, to the 5HT neurotoxicity produced by the Schedule I drug 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy"). That fenfluramine has long-lasting, possibly neurotoxic, effects upon central 5HT when administered in high doses has been reported by numerous laboratories (4, 10, 11, 23, 24). Nevertheless, the functional consequences of the serotonergic deficits are still unclear and the effect that large depletions of 5HT might have in humans exposed to fenfluramine over a long period of time is controversial (3).

The discriminative properties of fenfluramine have been well-established to exist in the rat by several investigators (9, 14, 22, 25). The results from these laboratories have suggested that fenfluramine acts as a drug capable of controlling differential responding in the discrimination paradigm by mediation of serotonergic neurons (14,25). It is the purpose of the present investigation to employ fenfluramine as the discriminative stimulus that will enable an animal to differentiate one response from another based solely upon the drug or nondrug condition under which it is placed. Subsequent to this training, a neurotoxic dose/regimen of fenfluramine will be administered to the animals in an effort to investigate the consequences of fenfluramine neurotoxicity upon its own discrimination.

METHOD

Subjects

Twelve male Sprague-Dawley rats were purchased from Zivic-Miller laboratories (Allison Park, PA) at a weight of 280–320 g and were housed individually in hanging wire cages. They were kept in a room maintained at a constant temperature (21–22°C) and humidity (50–65%) and illuminated 12 h per day (lights on at 0600 h). The rats were given water ad lib, but were on a restricted diet of commercial rat chow so as to maintain their body weights at approximately 85% of their free-feeding weights as determined by a growth chart from the supplier.

Apparatus

Twelve standard rodent operant test chambers (Lafayette Instrument Co., Lafayette, IN) were housed in light-proof, sound-attenuated and fan-ventilated outer shells. Each chamber was equipped with two levers mounted 7 cm apart and 2 cm above a grid floor. A food pellet receptacle was equidistant between the levers and was programmed to deliver a 45 mg Noyes food pellet as reinforcement. Located in an adjacent room, to control and record each training and test session, was solid-state programming equipment (Med Assoc., E. Fairfield, VT).

Shaping to Lever-Press Procedure

The food-deprived rats were administered distilled water (ve-

hicle) intraperitoneally (IP) 20 min prior to the start of the experiments and were trained to press either the right (n = 6) or the left (n = 6) lever to receive food reinforcement under a fixed ratio 1 (FR 1) schedule. Training continued as the FR schedule was made increasingly more demanding until an FR 10 schedule was achieved over a period of 7 days; this FR 10 schedule was maintained for 3 additional days. On the following training session, the rats received (IP) an equal volume (1 ml/kg) of the vehicle containing 2.0 mg/ml d,l-fenfluramine hydrochloride (calculated as base) 20 min prior to the training session. At that time, the rat was placed into a randomly assigned operant chamber and an FR 1 schedule on the opposite (the designated "drug-correct") lever was in effect. The FR schedule was gradually increased over a 5-day period until a stable FR 10 schedule was attained on the second lever and this schedule was maintained for 3 additional days.

Discrimination Training

Once FR 10 responding was achieved on both levers, the discrimination training phase began in which the food-motivated rats were required to press the fenfluramine-appropriate lever after fenfluramine administration and the vehicle-appropriate lever after vehicle administration in order to receive reinforcement. A biweekly repeating schedule of administration for either fenfluramine (F) or vehicle (V) injected (IP) at 20 min prior to beginning of the training session was in effect throughout the training phase: V-F-F-V-V, F-V-V-F-F. The lever pressed 10 times first was designated as the "selected" lever and every 10th press on the fenfluramine-correct lever was reinforced on days when the animals were administered fenfluramine, whereas every 10th response on the opposite lever was reinforced after vehicle administration. On any daily session, the animal received 40 food pellets after making 400 correct responses on the state-appropriate lever. Discrimination sessions were continued until each rat reached the performance criterion, i.e., selecting (pressing 10 times first) the appropriate lever, according to the state imposed, on 8 of 10 consecutive daily sessions. When this criterion was attained, the number of the first session of the 10 consecutive sessions was the measure referred to as the sessions-to-criterion [STC; (16)]. The animals were required to choose the state-appropriate lever on one additional set of 8 of 10 consecutive sessions; this measure constituted the sessions-to-criterion 2 (STC 2).

Dose-Response Relationship to Various Doses of Fenfluramine

After all the rats attained the training criterion and were, thus, judged able to discriminate between 2.0 mg/kg fenfluramine and its vehicle, the animals received various doses of fenfluramine (dose-response; DR) different from the training dose according to the following biweekly schedule: F-DR_1-V-DR_2-F, DR_2-V-DR_1-F-DR_3, etc., where F = fenfluramine training dose; V = vehicle; DR_1 = one other dose of F; DR_2 = second other dose of F, etc. Doses were administered IP at 20 min prior to testing and, on these test days, the animals were allowed to lever press until 10 responses were made on either lever. At that time, the rats were immediately removed from the operant test cage, without receiving reinforcement, and placed into their home cages in order to preclude any reinforcement (training) at a dose different than the 2.0 mg/kg fenfluramine dose used to train them. This procedure allowed calculation of the dose of fenfluramine that produced 50% discriminative performance, i.e., the ED_{50} value.

Saline and Fenfluramine Neurotoxic Regimen

After the dose-response determinations, a regimen of morning and afternoon IP administrations of saline was given over a 4-day period 10 days prior to redetermining the dose-response relationship. This regimen was intended to determine if saline administrations twice-a-day for 4 days, as well as the absence of training for 10 days, would affect discrimination of various doses of fenfluramine. Once this was determined, the next phase of the experiment was to administer a neurotoxic dose (6.25 mg/kg) of fenfluramine IP twice-a-day for 4 days, a regimen reported to produce serotonin neurotoxicity in animals sacrificed 14 days after its initiation (10).

TABLE 1

SCHEDULE OF ADMINISTRATION OF SALINE OR A HIGH, NEUROTOXIC DOSE (6.25 mg/kg) OF FENFLURAMINE (FENtx) AND TESTING OF DISCRIMINATION TO LOWER DOSES OF FENFLURAMINE (FEN)

Day	a.m. Series I/Series II	p.m. Series I/Series II
1	Saline/FENtx	Saline/FENtx
2	Saline/FENtx	Saline/FENtx
3	Saline/FENtx	Saline/FENtx
4	Saline/FENtx	Saline/FENtx
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.		
13	—	1.0 FEN
14	vehicle	2.0 FEN
15	—	0.5 FEN

Between these two experiments, i.e., after the dose-response experiments following repeated saline administration and prior to starting high dose fenfluramine injections, all rats were tested and retrained with two fenfluramine and two vehicle maintenance sessions. The regimens used after saline and fenfluramine are detailed in Table 1. Series I refers to the saline regimen and series II to the 6.25 mg/kg fenfluramine regimen of administrations over a 4-day period. In each case, the animals went without any training/testing until the 13th day when, in the afternoon, 1.0 mg/kg fenfluramine was tested in extinction. This was followed on day 14 with a nonreinforced vehicle test in the morning and a trial with 2.0 mg/kg fenfluramine (training dose) in the afternoon. Lastly, the afternoon session on the 15th day was conducted after administration of 0.5 mg/kg fenfluramine. In all of these four tests, the animal was immediately removed upon making 10 responses on either of the two levers.

Measurements and Statistics

The lever pressed 10 times was designated the "selected" lever. The percentage of rats selecting the lever appropriate for fenfluramine was the quantal measurement of discrimination. In addition, the number of lever-presses made upon the fenfluramine lever divided by the total number of responses on the fenfluramine and vehicle levers at the time that the tenth response is made on either lever, times 100, constitutes the quantitative measurement. The quantal data were analyzed by application of the method of Litchfield and Wilcoxon (12) which employs probits vs. log-dose effects, allows for the generation of ED_{50} values and tests for parallelism between dose-response curves. The quantitative data were calculated for each animal for each session, e.g., for an animal who pressed the fenfluramine lever ten times and the vehicle lever twice after the administration of fenfluramine,

TABLE 2

DOSE-RESPONSE EFFECTS WITH VARIOUS FENFLURAMINE DOSES AT 13, 14 AND 15 DAYS FOLLOWING MULTIPLE ADMINISTRATION OF EITHER SALINE OR 6.25 mg/kg FENFLURAMINE

	After Saline		After Fenfluramine	
Dose Fenfluramine (n)	Quantal	Quantitative (S.E.M.)	Quantal	Quantitative (S.E.M.)
2.0 (10)	90.0	84.4 (7.1)	90.0	80.7 (9.0)
1.0 (9)	88.9	80.1 (5.7)	100.0	91.4 (2.2)*
0.5 (11)	27.3	36.5 (10.5)	72.7	64.0 (9.6)*
0.0 (veh) (11)	18.2	26.5 (9.5)	18.2	31.2 (8.5)

n: number of rats (n = 11) responding at dose tested.
*$p<0.05$ (paired t-test).

the quantal (all-or-none) score would be fenfluramine as the selected lever, and the quantitative measurement would be fenfluramine lever responses divided by fenfluramine plus vehicle lever responses, or $10/10 + 2 = 0.833 \times 100$; thus, the quantitative measurement on this animal's test session would be 83.3%. The quantitative data were subsequently analyzed with a Student's paired t-test with $p<0.05$ set as the criterion for significance.

RESULTS

The rats rapidly learned to discriminate 2.0 mg/kg d,l-fenfluramine from its vehicle as they attained the session-to-criterion for the first time (STC 1) in a mean of 3.4 training sessions (range: 1–7 sessions). Likewise, the first session for the second 8 of 10 correct consecutive trials (STC 2) was, on average, the 13.4th training session (range: 11–17). Once all rats reached criterion performance, doses of fenfluramine lower than the training dose, i.e., 0.5, 1.0 and 1.5 mg/kg, were administered during test sessions. During these dose-response experiments, one of the 12 rats died of causes unrelated to the experiment and the results reflect this fact (n = 11). The training dose (2.0 mg/kg) of fenfluramine produced 100% of response (quantal measurement) upon the fenfluramine-appropriate lever. Likewise, 1.5 mg/kg fenfluramine also produced errorless discrimination. When 1.0 mg/kg was administered, 54.6% of first selected lever choices were on the fenfluramine-appropriate lever and with 0.5 mg/kg fenfluramine 36.4% of selected lever choices were made on this lever. Vehicle administration during interspersed trials produced 4.6% of selected lever choices on the fenfluramine lever or (to look at it a different way) 95.4% on the vehicle-appropriate lever. These results allowed for an ED_{50} value of 0.71 mg/kg (95% confidence limits: 0.52–0.95 mg/kg). Because of the similarity in discriminative performance after 1.5 and 2.0 mg/kg, the doses that were consequently selected to be used after the saline and neurotoxic fenfluramine regimen were 0.5, 1.0 and 2.0 mg/kg fenfluramine.

The results of testing these doses of fenfluramine after repeated treatment with saline and (later) a fenfluramine neurotoxic dose are presented in Table 2. When 1.0 mg/kg fenfluramine was tested on the afternoon of the 13th day (see Table 1, above), 9 of the 11 rats responded with 8 of them selecting the fenfluramine-appropriate lever; this yields a quantal measurement of 8/9 or 88.9% and a quantitative measurement of 80.1% was determined. On the next morning, vehicle administration resulted in all of the animals responding with two rats choosing the (inappropriate) fenfluramine lever. During the same day's afternoon test session, the training dose of 2.0 mg/kg fenfluramine resulted in 9 of 10 responding animals choosing the fenfluramine-correct lever. On the last day of postsaline testing, 0.5 mg/kg fenfluramine administered on the afternoon of the 15th day resulted in 3 of the 11 responding animals choosing the fenfluramine-appropriate lever.

Following a four-day period of retraining (see the Method section) in which the animals were observed to be capable of accurately discriminating fenfluramine from saline, they received 6.25 mg/kg fenfluramine twice-a-day for 4 days. The results of testing the same doses of fenfluramine 13, 14 and 15 days after initiating this regimen appear on the right side of Table 2. Nine of the 10 (90%) responding animals choose the fenfluramine lever after 2.0 mg/kg and all of the 9 responding animals (100%) choose this lever after 1.0 mg/kg fenfluramine. When 0.5 mg/kg was tested (on the 15th day), 8 of the 11 responding animals choose the fenfluramine-correct lever. When the quantitative measurements are compared following the fenfluramine neurotoxic regimen to these measurements following the repeated administrations of saline, there is a significant increase in discriminative performance after 0.5 and 1.0 mg/kg fenfluramine ($p<0.05$; paired t-test).

DISCUSSION

The results of this study demonstrate that the commercially available anorectic drug d,l-fenfluramine (Pondimin®) is capable of controlling differential operant responding in rats based on its discriminative stimulus properties. This has previously been reported by this (22) and numerous other laboratories (9, 14, 25). The acquisition of criterion performance, viz., two sets of 8 of 10 consecutive sessions in which the appropriate lever was pressed 10 times first, was accomplished in a mean of 13.4 sessions with a range of 11 to 17 sessions. This was slightly faster discriminative learning than previously reported (mean: 18.6 sessions) with the same fenfluramine training dose (22). Varying the dose of the training drug produced a stimulus generalization gradient which, when analyzed (12), yielded a dose of fenfluramine that would produce 50% fenfluramine-appropriate lever selections (the ED_{50} value) as 0.71 mg/kg, a value very similar to the $ED_{50} = 0.79$ mg/kg found in a previously trained group of animals (22).

When saline was administered in a volume of 1 ml/kg twice-a-day for 4 days and a dose-response relationship was determined starting 13 days after the initiation of the chronic regimen (see Table 1), the ED_{50} value ($= 0.60$ mg/kg) was within the 95% confidence limits (0.52–0.95) of the ED_{50} value generated prior to the 10-day hiatus without continued training. Thus, the regimen of saline administration, as well as the "time-off," did not significantly affect the animals' discrimination of the training or lower doses of fenfluramine. In contrast, the administration of 6.25 mg/kg fenfluramine twice-a-day for 4 days produced a significant increase in the quantitative discrimination measurement after 0.5 and 1.0 mg/kg fenfluramine when these doses of fenfluramine were tested 13 and 15 days, respectively, after the initiation of the fenfluramine neurotoxic regimen. The quantal ED_{50} value for the three doses of fenfluramine tested after this neurotoxic regimen was 0.009 mg/kg; the potency ratio between the dose-response generated after saline compared to that measured after fenfluramine neurotoxic regimen was 6.89; significant at $p<0.05$ (12).

By employing the same dose and injection schedule previously used in an examination of the ability of fenfluramine to significantly deplete levels of 5HT in various brain areas (10), an increase in discriminative performance to fenfluramine was observed. Thus, at a time when repeated injections of fenfluramine have been shown to produce as much as an 80% depletion of 5HT in specific brain areas, rats were shown to have an increase in their fenfluramine-induced discriminative stimuli that resulted in a significant increase in discrimination of half and one-quarter of the training dose (Table 2), as well as a significant shift of the

dose-response curve to the left. The most parsimonious explanation for this heightened sensitivity resides in the possible development (after repeated fenfluramine administrations and over time) of postsynaptic receptor supersensitivity following chronically decreased 5HT release (11). Indeed, this process has been suggested to explain the restoration of normal function and the tolerance observed to the anorectic action of fenfluramine (21). It is generally thought that chronic treatment with drugs that promote 5HT neurotransmission create "down regulation" or a decreased number of receptor sites. However, results of the present study are consistent with the hypothesis that fenfluramine may be neurotoxic to presynaptic structures. In this case, posttesting with fenfluramine would result in an increase in discriminative performance compatible with hypersensitivity of postsynaptic receptors. This hypothesis is supported by recent reports (1, 15, 17) which demonstrate profound fenfluramine-related degeneration of 5HT-immunoreactive axons in rat cortex and striatum. The present study demonstrated the functional increase in fenfluramine-trained animals' ability to discriminate fenfluramine following a regimen that has repeatedly been shown to cause serotonergic neuronal degeneration.

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