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Norfenfluramine, the Fenfluramine Metabolite, Provides Stimulus Control: Evidence for Serotonergic Mediation

JOHN W. BOJA[1] AND MARTIN D. SCHECHTER[2]

Department of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272

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BOJA, J. W. AND M. D. SCHECHTER. *Norfenfluramine, the fenfluramine metabolite, provides stimulus control: Evidence for serotonergic mediation.* PHARMACOL BIOCHEM BEHAV 31(2) 305–311, 1988.—Nine male rats were trained to discriminate 1.4 mg/kg norfenfluramine (NF) from its vehicle using a two-lever, food-motivated, operant discrimination task. Once trained, the rats showed a dose-dependent decrease in responding on the NF-correct lever following decreased doses of NF (ED_{50}=0.71 mg/kg). Administration of 2.0 mg/kg fenfluramine (FEN) produced 100% responding on the NF-correct lever and decreasing doses of FEN, likewise, produced a dose-dependent decrease in responding on the NF-correct lever (ED_{50}=1.30 mg/kg). Time-course data indicated that NF has a fast onset and a peak effect at 20–60 min after administration. Analysis of the time-course data provided a half-life of approximately 8 hr. In contrast, FEN did not show the rapid onset that was observed with NF. However, NF had a similar peak effect and half-life. These results indicate a pharmacological similarity between NF and FEN. However, the difference in onset of action suggests a possible difference between the parent drug and its metabolite. The serotonergic agonists mCPP, DOI, 5-MeODMT and LSD generalized to 1.4 mg/kg NF, whereas neither TFMPP nor 8-OHDPAT generalized to NF. The dopaminergic agonist AMPH also did not generalize to NF. The implications of these findings are discussed.

Drug discrimination Rats Serotonin Norfenfluramine Fenfluramine Time-course

NORFENFLURAMINE (NF) is the pharmacologically active N-dealkylated first metabolite of the anorectic drug fenfluramine (FEN) (1, 2, 5, 11, 25). Fenfluramine has been shown to enhance serotonin (5-HT) neurotransmission by releasing 5-HT from the nerve terminal and by inhibiting its reuptake (11,27). Like its parent compound, NF has been shown to enhance 5-HT neurotransmission. However, NF displays a greater potency (16) and its site and/or mechanism of action may differ from that of FEN, i.e., FEN is thought to produce increased 5-HT accumulation by release from the granular pool and uptake inhibition, whereas NF may release 5-HT from a extravesicular 5-HT pool (3,4). In addition, the plasma half-life of NF has been reported to be longer than that of FEN (6,7).

The discriminative properties of FEN have been well established by several investigators (16, 21, 31, 39), and a greater potency of NF was demonstrated in rats trained to discriminate 3 mg/kg FEN from vehicle in a two-lever drug discrimination task, i.e., 2 mg/kg NF produced discriminative performance (generalization) similar to the higher training dose of FEN (21). In contrast, NF has never been used to train animals in the drug discrimination procedure and in light of the statement that "it remains unclear whether the pharmacologically active agent after administration of FEN is the parent compound itself or its dealkylated metabolite norfenfluramine" (21), an investigation employing NF as the training drug may provide a more accurate assessment of the discriminative cue produced by its parent compound. The use of NF will also bypass the necessary metabolic conversion of FEN to NF in rats and, thus, may provide a more accurate estimate of the time-course and potency of the FEN cue.

Biochemical studies have shown that there is a heterogeneous population of 5-HT receptors characterized as either the 5-HT_1 receptor, which is labeled by [H^3]-5-HT, or 5-HT_2, which is labeled by [H^3]spiperone binding (23,29). The 5-HT_1 receptor subtype has been further characterized as to either a 5-HT_{1A} or 5-HT_{1B} by the position on an antagonist curve (29,34). The 5-HT_{1A} sites are characterized by both high affinity binding of both 5-HT and 5-HT antagonists, while 5-HT_{1B} receptors show high affinity 5-HT binding and low affinity 5-HT antagonist binding.

[1]Present address: National Institute on Drug Abuse, Addiction Research Center, P.O. Box 5180, Baltimore, MD 21224.
[2]Requests for reprints should be addressed to Martin D. Schechter.

To date there has been only a published abstract (35) regarding evidence of any specific 5-HT mediation of the discriminative properties of FEN. This paucity of evidence may have been largely due to lack of specific 5-HT receptor agonists or antagonists. With the recent advent of specific agonists for the several subtypes of 5-HT receptors including: 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT), a 5-HT_{1A} agonist (34,38), 1-(m-trifluoromethylphenyl)piperazine (TFMPP), a 5-HT_{1B} agonist (22, 23, 32, 34), 1-(2,5-dimethoxyphenyl-4-iodo)-2-aminopropane (DOI), a 5-HT_2 agonist (12, 15, 31), and pirenperone, a 5-HT_2 antagonist (9,26), this is currently possible.

The purpose of the experiments detailed herein was to determine what differences may exist between FEN and NF and to determine which 5-HT receptor subtypes may mediate this behavioral effect of NF.

METHOD

Subjects

Nine male Sprague-Dawley (Zivic-Miller) rats weighing 320–450 g at the beginning of the experiment were the subjects used. The rats were individually housed in galvanized cages with free access to water, except during experimental sessions, and were maintained at 80% of their free-feeding body weight. The rats were trained 5 days per week at the same time of day (1300–1400 hr).

Apparatus

Nine standard rodent operant test cages (Lafayette Instrument Co., Lafayette, IN) were equipped with two levers mounted 7 cm above the metal grid floor and 7 cm apart. Equidistant between the two levers and 2 cm above the floor was located a food pellet receptacle. The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and a 9 watt houselight. Solid-state programming equipment (Med Assoc., E. Fairfield, VT) was used to control and record each session and was located in an adjacent room.

Shaping to Lever-Press Procedure

The food-deprived rats were administered vehicle (distilled water) intraperitoneally (IP) 20 min prior to the start of the experiment and were trained to press either the right (N=5) or left (N=4) lever to receive a food reinforcement (45 mg Noyes food pellet) under a fixed-ratio 1 (FR 1) schedule. Training continued as the FR schedule was gradually increased to FR 10 over a period of 6 days; this FR 10 schedule was maintained for 3 days. On the following training session, the rats received (IP) an equal volume (1 ml/kg) of vehicle containing 1.4 mg/ml NF 20 min prior to the test session. The rats were then placed on an FR 1 schedule on the opposite (the drug-correct) lever. The FR schedule was gradually increased over a 4 day period until a stable FR 10 was attained; this schedule was maintained for 3 days.

Discriminative Training

Subsequently, the following biweekly treatment schedule was instituted with either drug (D) or vehicle (V) administered 20 min prior to the beginning of the training session: V-D-D-V-V, D-V-V-D-D. The lever pressed 10 times first was designated as the "selected" lever. The training criterion was achieved when an animal first chose the appropriate lever according to the state imposed, i.e., drug or vehicle, on 16 daily sessions out of a total of 20 consecutive sessions. This 16 out of 20 criterion was required to be performed before any further testing was conducted. The number of sessions this required constituted the sessions-to-criterion (STC) (28).

Dose-Response of NF and FEN at 20 Min

After all the rats had met the training criterion and were, thus, judged able to discriminate NF from its vehicle, the animals received various doses of NF (dose-response, DR) according to the following weekly schedule: NF-DR_1-V-DR_2-NF, DR_2-V-DR_1-NF-DR_3, etc.; where NF=norfenfluramine training dose; V=vehicle; DR_1=one lower dose of NF; DR_2=second dose of NF. All 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 had been recorded on either lever. The rats were immediately removed from the operant test cages, without receiving reinforcement, and placed into their respective home cages in order to preclude any continued training at a dose other than the (1.4 mg/kg) NF dose used in training.

Following the dose-response experiments with NF, substitution (generalization) tests with FEN were conducted according to the above schedule for dose-response. Thus, each dose of FEN was preceded both by a vehicle or 1.4 mg/kg NF maintenance session. This counterbalanced design detects and corrects for any possible carry-over effect from the previous days's (drug) effects. Doses of 1.0, 1.5 and 2.0 mg/kg FEN were each tested on two occasions in each of the nine rats.

Time-Course of NF and FEN Action

To determine the time-course of the NF discriminative cue, rats were injected (IP) with 1.4 mg/kg NF, returned to their home cage and allowed to remain there for varying intervals from 10 to 960 min before being placed into the test chambers. The order in which the various time-delays after NF administration were tested was randomized between subjects such that each rat received each postinjection time interval delay twice, with each time preceded by one maintenance session with 1.4 mg/kg NF and one vehicle tested at 20 min postinjection. Animals failing to maintain discrimination in these maintenance trials were to be eliminated from the study. However, this did not occur. The half-life ($T_{1/2}$) of NF was determined by linear regression from the terminal linear part of the time-course curve generated from this experimentation.

To determine the time-course of the generalization to the FEN cue in the NF-trained rats, the dose of FEN found to produce maximal generalization in substitution tests (2.0 mg/kg) was administered to rats and, like NF, was tested in intervals from 10 to 960 minutes later. As with the NF time-course determinations, maintenance sessions with NF and vehicle at 20 min postinjection were interspersed between FEN postinjection sessions.

Dose-Response Testing of NF and FEN at 10 Min

Observations that the maximal difference between the discriminative effects of NF and FEN occurred at 10 min postadministration (see Results) led to a series of experiments in which various doses of each drug were tested at a fixed time postinjection, i.e., at 10 min. Testing at 10 min for

both NF and FEN was performed in a manner identical to that done at 20 min postinjection, as described above.

Stimulus Generalization Studies

Tests of stimulus generalization commenced with various doses of other agents in order to determine if those agents produced stimulus effects that would be recognized as similar or dissimilar to the stimulus produced by NF. Testing was carried out according to the following repeating 2 week schedule; NF-SG_{D1}-V-SG_{D2}-NF, SG_{D2}-V-SG_{D1}-NF-SG_{D3}, etc., where NF=1.4 mg/kg NF, V=vehicle, SG_{D1}=stimulus generalization dose 1, SG_{D2}=stimulus generalization dose 2, SG_{D3}=stimulus generalization dose 3, etc. Generalization was said to occur in these animals if responding on the NF-appropriate lever was 80% or above. This seemed appropriate as the original training criterion was the same 80% as described above.

Stimulus Antagonism Studies

Tests of stimulus antagonism were conducted with the NF/vehicle discrimination maintained throughout, using the specific 5-HT_2 antagonist pirenperone (PIR). PIR was administered 20 min prior to administration of 1.4 mg/kg NF (the training dose) and the animals were tested 20 min following the administration of the training drug. Stimulus antagonism was said to occur in the animals if, after administration of a given dose of antagonist prior to the administration of 1.4 mg/kg NF, NF-appropriate responding was reduced to 20% or less (the criterion for vehicle-appropriate responding).

Measurements and Statistics

The lever pressed 10 times first was designated the "selected" lever. The percentage of rats selecting the lever appropriate for NF was the quantal measurement of discrimination and quantal data are presented as percent correct first choices on the NF lever. In addition, the number of lever presses on the NF-correct lever divided by the total number of responses on both levers prior to 10 responses on either lever, times 100, constitutes the quantitative measurement. Both measurements were utilized as suggested previously (37). Quantal data were compared by the method of Litchfield and Wilcoxon (20) which employs probit vs. log-dose effects, allows for the generation of ED_{50}'s and can test for parallelism between dose-response curves. Quantitative data were compared by a two-tailed paired t-test of means ($p<0.05$).

Drugs

The drugs (abbreviation, supplier) used in this study were: d,l-norfenfluramine HCl (NF; A. H. Robins, Richmond, VA), d,l-fenfluramine HCl (FEN; A. H. Robins), 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT; RBI, Research Biochemical Inc., Natick, MA), 1-(m-trifluoromethylphenyl)piperazine (TFMPP; RBI), 1-(2,5-dimethoxyphenyl-4-iodo)-2-aminopropane (DOI; RBI), m-chlorophenylpiperazine HCl (m-CPP; RBI), pirenperone (PIR; Janssen Pharmaceutics, Beerse, Belgium), 5-methoxy-N,N-dimethyltryptamine hydrogen oxalate (5-MeODMT; RBI), d-amphetamine HCl (AMPH; Sigma, St. Louis, MO). All drugs were dissolved in deionized water and were injected IP in a constant volume of 1 ml/kg. NF, FEN and d-amphetamine doses were calculated as the salt, all others were calculated as the free base.

TABLE 1

DISCRIMINATION OF VARIOUS DOSES OF NF, AND GENERALIZATION OF FEN, IN RATS (n=9) TRAINED TO DISCRIMINATE 1.4 mg/kg NF FROM VEHICLE AND TESTED AT 20 MIN POSTINJECTION

Dose (mg/kg)	Quantal	Quantitative (S.D.)
1.4 NF	92.2	89.5 (10.1)
0.7 NF	55.0	52.7 (1.8)
0.35 NF	16.7	21.9 (9.0)
0.175 NF	0.0	6.3 (0.0)
0.0 (V)	0.0	2.8 (2.1)
ED_{50} (mg/kg)	0.71	0.59
(95% conf. limit)	(0.14–3.60)	(0.37–0.96)
2.0 FEN	100.0	88.9 (6.9)
1.5 FEN	72.2	62.5 (23.7)
1.0 FEN	16.7	18.2 (7.4)
ED_{50} (mg/kg)	1.30	1.23
(95% conf. limit)	(1.08–1.58)	(1.09–1.66)

Parallelism: Quantal—critical $t=3.18$ > calculated $t=0.38$. Quantitative—critical $t=3.18$ > calculated $t=0.35$.

RESULTS

Discriminative Learning

The rats rapidly learned to discriminate 1.4 mg/kg NF from vehicle. The sessions-to-criterion (STC), i.e., when 16 sessions correct out of 20 consecutive sessions were initially reached, was attained in a mean of 20.2 (±1.1) sessions, with a range of 18–22 sessions. Thus, all rats were judged to be able to correctly discriminate 1.4 mg/kg NF from its vehicle by the 22nd session (11 sessions with vehicle and 11 sessions with 1.4 mg/kg NF). Once the criteria was attained the rats were judged capable of correctly discriminating 1.4 mg/kg NF from vehicle and further testing commenced.

Dose-Response to NF and FEN at 20 Min

Maintenance sessions with 1.4 mg/kg NF produced 92.2% of "selected" lever (quantal) responding on the NF-correct lever, whereas vehicle administration produced no responding on this lever (or 100% responding on the vehicle-correct lever) as presented in Table 1. Decreasing doses of NF produced decreased responses on the NF-correct lever both in terms of the quantal and quantitative measurement. Analysis of the quantal dose-response relationship (20) yielded an ED_{50} (with 95% confidence limits) of 0.71 (0.14–3.60) mg/kg and generated an ED_{50} of 0.59 (0.37–0.96) mg/kg for the quantitative data.

Administration of 2.0 mg/kg of FEN to the NF-trained rats resulted in 100% of the first-choice (quantal) responses on the NF-appropriate lever, as presented in the bottom part of Table 1. Decreasing doses of FEN, likewise, produced decreased selections of the NF-correct lever and a quantal dose-response curve which when compared (20) to the dose-response relationship produced by various doses of NF was shown to be parallel (critical $t=3.18$ > calculated $t=0.38$). Analysis of the dose-response data for FEN yielded a quantal ED_{50} (with 95% confidence limits) of 1.30 (1.08–

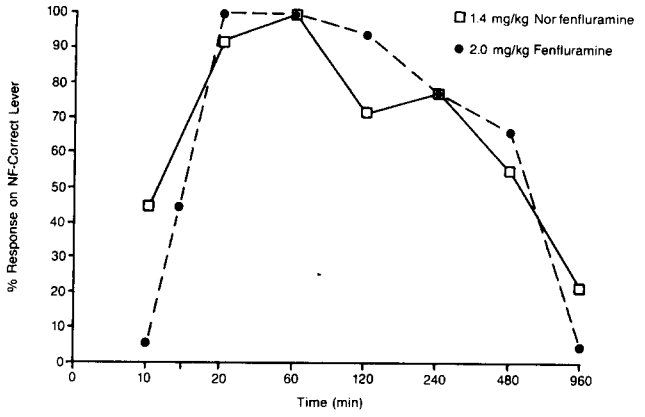

FIG. 1. The NF-appropriate quantal discriminative response to either 1.4 mg/kg NF or 2.0 mg/kg FEN following various time-to-testing intervals. Ordinate: percent of rats (n=9) selecting the NF-appropriate lever; abscissa: postadministration time in minutes.

1.58) mg/kg, and a quantitative ED_{50} of 1.23 (1.09–1.66) mg/kg. Comparing ED_{50}'s of FEN:NF results in a quantal ratio of 1.30:0.71 and a quantitative ratio of 1.23:0.59; hence, NF is approximately twice as potent as FEN.

Time-Course of Action for NF and FEN

As seen in Fig. 1, the onset of the NF cue is rapid, i.e., 44.5% quantal NF responses in 10 min, and long lasting, 55.6% quantal responses after 480 min. The NF discriminative performance was observed to be maximal between 20–60 min, and decreased to 72.2% at 120 min following NF administration. After 960 min, the percent responding (quantitative measurement) on the NF-correct lever is not significantly different from vehicle ($t=0.99$, $p=0.17$). The calculated half-life ($T_{1/2}$) for 1.4 mg/kg NF is approximately 8 hr.

The onset of action of the FEN cue in NF-trained rats is not as rapid as the onset of the NF cue, i.e., 5.6% quantal responses 10 min following administration and 44.4% quantal responses after 15 min (Fig. 1). However, at 20 min following FEN administration the effect upon discriminative performance is sufficient to produce 100% quantal NF-appropriate responding. Similar to the results after NF administration, peak effect with FEN occurred between 20 and 60 min and the percent responding on the NF-correct lever after 960 min following FEN administration is not significantly different from vehicle responding. The calculated $T_{1/2}$ for 2.0 mg/kg FEN is similar to that of NF, i.e., approximately 8 hours.

Dose-Response to NF and FEN at 10 Min

Varying doses of NF administered 10 min prior to testing resulted in a typical dose-response relationship; analysis of the dose-response data at 10 min generated by decreasing doses of NF revealed a ED_{50} (with 95% confidence limits) of 1.13 (0.80–1.58) mg/kg for quantal data and a similar ED_{50} of 1.14 (0.39–3.26) mg/kg using the quantitative measurement (Table 2). Likewise, testing at various doses of FEN at 10 min yielded a quantal ED_{50} (with 95% confidence limits) of 2.80 (2.47–3.17) mg/kg and a quantitative ED_{50} of 2.82 (2.34–3.40) mg/kg. The quantal dose-response curve produced by analysis of the NF data at 10 min was parallel to the dose-response curve produced by NF at 20 min (critical $t=2.78$ > calculated $t=2.09$). Furthermore, the FEN dose-response curve at 10 min was parallel to both the FEN dose-response curve at 20 min (critical $t=3.18$ > calculated $t=2.65$) and the NF dose-response curve at 10 min (critical $t=2.78$ > calculated $t=0.82$). However, at ten min the ED_{50} ratio of FEN:NF is slightly different than that seen at 20 min.

TABLE 2

DISCRIMINATION OF VARIOUS DOSES OF NF, AND GENERALIZATION OF FEN, IN RATS (n=9) TRAINED TO DISCRIMINATE 1.4 mg/kg NF FROM VEHICLE AND TESTED AT 10 MIN POSTINJECTION

Dose (mg/kg)	Quantal	Quantitative (S.D.)
2.1 NF	93.3	86.4 (4.9)
1.4 NF	100.0	89.7 (2.8)
0.7 NF	27.8	30.8 (11.7)
0.35 NF	0.0	5.7 (3.5)
0.0 (V)	0.0	3.2 (1.9)
ED_{50} (mg/kg)	1.13	1.14
(95% conf. limit)	(0.80–1.58)	(0.39–3.26)
4.0 FEN	94.1	81.5 (10.5)
3.0 FEN	52.9	48.6 (51.6)
2.5 FEN	44.4	43.6 (43.8)
2.0 FEN	5.6	19.0 (14.0)
ED_{50} (mg/kg)	2.80	2.82
(95% conf. limit)	(2.47–3.17)	(2.34–3.40)

Parallelism: Quantal—critical $t=2.7$ > calculated $t=2.0$. Quantitative—critical $t=2.7$ > calculated $t=1.7$.

Stimulus Generalization Studies

The results of the stimulus generalization studies are presented in Table 3. 5-MeODMT (0.5–3.0 mg/kg) was observed to generalize to the 1.4 mg/kg NF cue in a dose-related manner. The 3.0 mg/kg dose of 5-MeODMT produced 94.4% quantal and 87.8% quantitative responding on the NF-appropriate lever. Probit analysis of the 5-MeODMT dose-response curve yielded a quantal ED_{50} (with 95% confidence limits) of 1.40 (0.91–1.82) mg/kg and a quantitative ED_{50} of 1.50 (1.06–2.12) mg/kg. Analysis of the slope of the 5-MeODMT-produced dose-response curve and the previously determined dose-response curve for NF indicated that these curves are parallel within statistical limitations (critical $t=3.2$ > calculated $t=1.85$).

Likewise, mCPP was observed to generalize with the administration of 1.0 mg/kg mCPP producing 94.4% and 81.4% quantal and quantitative responding, respectively, on the NF-appropriate lever. Similar analysis of the mCPP dose-response curve yielded an ED_{50} of 0.40 (0.25–0.63) mg/kg for the quantal measurement and 0.57 (0.34–0.96) mg/kg for the quantitative measurement. Comparison between the mCPP dose-response curve and the NF dose-response curve, again, indicated that the two curves were parallel (critical $t=3.2$ > calculated $t=0.5$) within statistical limits.

The highest dose of DOI administered (1.0 mg/kg)

TABLE 3

GENERALIZATION OF VARIOUS SEROTONERGIC AND DOPAMINERGIC AGENTS TO THE NF-APPROPRIATE LEVER IN RATS TRAINED TO DISCRIMINATE 1.4 mg/kg NF FROM VEHICLE

Treatment	Dose (mg/kg)	Quantal	Quantitative (SD)
5-MeODMT	3.0	94.4	87.8 (0.6)
	2.0	77.8	69.1 (13.6)
	1.0	38.9	36.6 (18.2)
	0.5	0.0	0.6 (0.8)
mCPP	1.0	94.4	81.4 (13.5)
	0.5	44.4	29.6 (25.2)
	0.25	33.3	19.9 (26.5)
DOI	1.0	88.9	71.4 (10.7)
	0.5	72.2	65.8 (18.9)
	0.25	27.8	31.0 (0.6)
LSD	0.06	83.3	72.7 (9.3)
	0.03	16.7	24.3 (0.3)
TFMPP	3.0	50.0	52.1 (11.2)
	2.5	72.2	56.8 (0.1)
	2.0	55.6	52.2 (7.4)
	1.5	50.0	48.3 (7.4)
	1.0	44.4	46.4 (13.5)
8-OH-DPAT	0.8	64.7	58.7 (3.7)
	0.6	59.1	52.8 (0.6)
	0.4	66.7	56.5 (0.2)
	0.25	27.8	34.5 (4.5)
AMPH	1.6	0.0	8.2 (1.3)
	1.2	5.6	11.1 (2.8)
	0.8	11.1	15.6 (19.6)
	0.4	5.6	13.7 (11.9)

produced 88.9% quantal responding and 71.4% quantitative responding on the NF-appropriate lever. Probit analyses of the DOI dose-response data resulted in an ED_{50} of 0.37 mg/kg (0.22–0.60 mg/kg) for the quantal measurement and 0.41 mg/kg (0.20–0.82 mg/kg) for the quantitative measurement. Analysis of the slopes for DOI and that for NF indicated that the two curves were parallel (critical $t=3.2$ > calculated $t=0.02$).

LSD (0.06 mg/kg) produced 83.3% quantal responding and 72.2% quantitative responding on the NF-appropriate lever. Limited quantities of LSD prevented testing at more doses. However, analysis of the slopes of the LSD curve (using the two data points) and the NF curve indicated that the parallelism of the slopes for the two curves was significantly different (critical $t=4.3$ < calculated $t=5.3$).

In contrast to these generalizations, neither TFMPP nor 8-OH-DPAT completely generalized to NF. Although the 2.5 mg/kg dose of TFMPP produced 72.2% and the 0.8 mg/kg dose of 8-OH-DPAT produced 64.7% responding on the NF-lever, higher doses of each were precluded by the appearance of behavioral disruption, i.e., extensive delays between placement into the test chamber and the onset of lever pressing.

No dose of AMPH (0.4–1.6 mg/kg) generalized to the NF cue. The greatest response on the NF-appropriate lever produced by AMPH (0.8 mg/kg) was only 11.1% quantal and 15.6% quantitative, both well below the 20% criterion of vehicle-like responding. Higher doses of AMPH produced even less responding on the NF-appropriate lever.

TABLE 4

EFFECT OF VARIOUS DOSES OF THE SEROTONERGIC ANTAGONIST PIRENPERONE UPON NF DISCRIMINATION IN RATS TRAINED TO DISCRIMINATE 1.4 mg/kg NF

Pretreatment*	Dose (mg/kg)	Treatment	Dose (mg/kg)	Quantal	Quantitative (SD)
Pirenperone	0.04	NF	1.4	83.3	80.9 (16.8)
	0.08		1.4	66.7	57.9 (30.2)
	0.16		1.4	16.7	26.6 (27.4)
	0.32		1.4	16.7	18.3 (21.2)

*Pirenperone was administered IP 20 min prior to NF and all rats (n=9) were tested 20 min following the second injection.

Stimulus Antagonism

Pirenperone (PIR; 0.04–0.32 mg/kg) antagonized 1.4 mg/kg of NF in a dose-responsive manner as presented in Table 4. Administration of 0.16 mg/kg PIR 20 min prior to the administration of 1.4 mg/kg NF reduced NF-like responding to 16.7% quantal and 26.6% quantitative. Increasing the dose of PIR to 0.32 mg/kg produced no further reduction in quantal responding on the NF-appropriate lever, but further reduced quantitative responding to 18.3%.

DISCUSSION

Fenfluramine (FEN) is an anorectic drug which is first, and mainly, metabolized to an active metabolite norfenfluramine (NF) (1, 2, 5, 11, 25). Both FEN and NF have been found to be present in plasma and brain tissue of the rat following FEN administration (11), as well as in the urine of humans (1, 2, 25). Brain levels of both FEN and NF are several times higher than analogous plasma levels (6, 7, 9). In addition, the half-life of both FEN and NF is longer in the brain than in the plasma (6,9). The present study reports that NF, like its parent compound FEN, can serve as a drug capable of controlling discriminative behavior in the rat. The dose of NF utilized (1.4 mg/kg) produces no apparent anorectic effects as indicated by the animals' continued willingness to lever-press for food reward. In addition, repeated administration of this dose did not produce any apparent tolerance to NF as demonstrated by lack of a change in sensitivity to NF during the interspersed NF maintenance sessions.

The discrimination of NF was shown to be dose-responsive. The ED_{50} for NF (0.71 mg/kg) was approximately one-half the training dose, as was previously shown to be the case with FEN at a training dose of either 3.0 (16,21) or 1.0 mg/kg (39). The dose-response curves of NF and FEN are parallel, within statistical limits (20), suggesting that the two drugs may be acting via a common mechanism (19).

The ED_{50} calculated for FEN as it generalized from NF was 1.30 mg/kg or approximately 1.8 times greater than that of NF for the quantal measurement and 1.23 mg/kg or 2.1 times that of NF for the quantitative measurement. These ratios approximate the 1.5 times greater potency of NF as compared to FEN in FEN-trained rats (16,21). Furthermore, the ED_{50} of d-fenfluramine was 1.5 times the ED_{50} of NF

required for the same anorectic effect in rats (4) and the doses required for inhibiting 5-HT uptake, 5-HT binding or stimulating 5-HT release were greater for FEN than NF by factors of 2.8, 5.0 and 1.7, respectively (24).

The optimal discrimination detection of either the NF or FEN interoceptive cue occurs between 20 and 60 min postinjection (Fig. 1). The calculated $T_{1/2}$ for both compounds (8 hours) corresponds to a previously calculated brain level $T_{1/2}$ of 8 hours following IV administration of 20 mg/kg FEN in rats (36).

The parallelism of both the NF and FEN curves at either 10 and 20 min suggests that both NF and FEN are acting via the same mechanism (19). It would seem unlikely, however, that FEN could be metabolized within 10 min to NF levels high enough to produce NF-correct responding; this suggests that both NF and FEN may each be capable of serving as the discriminative cue. Since NF is the active metabolite of FEN there may be a subtle difference in the pharmacodynamics and, thereby, the potencies between NF, when given directly to the animals, and the NF which was derived from injected FEN. Furthermore, a difference in basicity may exist between NF and FEN in that, by being a primary amine, NF is a weaker base than the secondary amine FEN. At the present time it still cannot be determined if NF is the discriminative cue following FEN administration. However, Young and Glennon (41), after observing NF is 1.5 to 2.0 times as potent as FEN in a similar behavioral task, suggested that "norfenfluramine probably exerts a major role in the discriminative stimulus profile of fenfluramine."

The existence of several 5-HT receptor subtypes has been well established (23, 29, 33). If the mechanism of action for NF is, indeed, via the release of endogenous 5-HT, then the released 5-HT should interact at all 5-HT receptor subtypes nonselectively. Through the use of both specific and nonspecific 5-HT agonists the exact nature of the NF cue can be determined.

The discriminative stimulus properties of 5-MeODMT have been extensively examined (13, 14, 42, 43) and it was concluded that this compound possesses both 5-HT_1 and 5-HT_2 agonist properties. This conclusion is in agreement with receptor binding studies that describe 5-MeODMT as a nonselective 5-HT agonist (42,43). 5-MeODMT has been shown to transfer to rats trained to discriminate 1.5 mg/kg FEN from saline, ED_{50}=7.19 mg/kg (35). Due to the similarity of NF to FEN it was, therefore, very likely that 5-MeODMT would have also transferred to NF. The ability of NF to generalize to 5-MeODMT may involve its ability to release 5-HT, which in turn stimulates the 5-HT receptors in a nonselective manner. It may be this nonselective stimulation of the 5-HT receptor subtypes that prevents the generalization of the specific 5-HT subtype agonists. Hence, 8-OHDPAT fails to generalize to NF since it only stimulates the 5-HT_{1A} receptor (34,38). The same holds true for TFMPP since it has been shown to be a 5-HT_{1B} agonist (22,34), it only stimulates one receptor out of the many that NF could possibly stimulate. Neither of these compounds generalized to FEN-trained animals either (35).

Conversely, mCPP generalized to NF and to FEN (ED_{50}=0.56 mg/kg) (35). The ability of mCPP to generalize to NF while TFMPP did not may reside in the fact that while both compounds are 5-HT_1 selective, TFMPP is more selective for the 5-HT_1 receptor than is mCPP (23). The 5-HT_1/5-HT_2 selectivity ratio of mCPP is approximately 4 times that of TFMPP, i.e., TFMPP is 4 times as selective for the 5-HT_1 receptor than mCPP.

Turning to a different area of interest, 5-MeODMT has been shown to be hallucinogenic in man. The hallucinogenic nature of 5-MeODMT was also demonstrated in the discriminative stimulus paradigm, as 5-MeODMT transferred to both DOM (13) and LSD (42). Since the activation of the 5-HT_2 receptor is thought to be intimately involved with hallucinatory behavior (14), activation of the 5-HT_2 receptor by NF could explain the hallucinatory side effects that have been reported following high doses of FEN in humans (17,30).

NF also transferred to both DOI (the iodinated form of DOM) and LSD. DOM has been shown to be a very selective agent for the 5-HT_2 receptor site (15). The discriminative profile of DOI also demonstrates a 5-HT_2 selectivity (12). FEN has been shown to partially generalize for LSD (40). Conversely, 0.06 mg/kg LSD produced only partial generalization in rats trained to discriminate FEN (21). The ability of NF to generalize to LSD to a greater extent than FEN may reside in the increased ability of NF to stimulate the 5-HT_2 receptor.

Further proof for the role of the 5-HT_2 receptor in the interoceptive cue of NF is the ability of the 5-HT_2 antagonist pirenperone (PIR) to block the NF cue. Administration of 0.16 mg/kg PIR reduced NF-like responding following NF administration to 16.7%, while the same dose of PIR only reduced FEN-like responding in FEN-trained animals to 50% following administration of the training dose of FEN (31). This last result must be viewed with caution, since the reduction in drug-like responding following PIR may be strain-dependent. In the forementioned study PIR failed to reduce FEN-responding in a different strain (31).

In conclusion, the ability of NF to generalize to FEN, 5-MeODMT and mCPP appears to be dependent upon its ability to release endogenous 5-HT. The released 5-HT is subsequently able to interact at each 5-HT receptor subtype. The ability of NF to generalize to agents such as DOI, LSD and to a lesser extent 5-MeODMT may reside in its ability to stimulate the 5-HT_2 receptor. These generalizations with the specific 5-HT_2 agonists and the attenuation of the NF cue by the specific 5-HT_2 antagonist PIR, suggests that it is mainly by activation of the 5-HT_2 receptor site that NF provides stimulus control. It is unclear at this time whether this is a direct postsynaptic effect, as previously suggested (18), or an indirect effect with NF releasing 5-HT preferentially in a brain area rich in 5-HT_2 sites.

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