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Effect of Fenfluramine and Nicotine Upon A Stimulant-Depressant Continuum

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SCHECHTER, M. D. *Effect of fenfluramine and nicotine upon a stimulant-depressant continuum.* PHARMAC. BIOCHEM. BEHAV. 15(3) 371–375, 1981.—Rats were rapidly trained to discriminate between 0.8 mg/kg *d*-amphetamine and 6 mg/kg pentobarbital in a two-lever food-motivated operant task by imposing the drug states from the earliest stage of training. Once trained, rats were administered lower doses of each of the training drugs and both *d*-amphetamine and pentobarbital were observed to produce dose-responsive effects upon discriminative performance. When graphically represented, the dose-response curves were shown to be parallel suggesting a common site and/or mechanism of action. Administration of fenfluramine (1.5 and 2.25 mg/kg) produced pentobarbital-appropriate responding, whereas the injection of three doses of nicotine (0.1–0.4 mg/kg) resulted in amphetamine-like discriminative responding. Inspection of dose-response curves suggested that fenfluramine produces its pentobarbital-like effects by acting differently than does pentobarbital and, although nicotine produces amphetamine-like effects, it acts by a different mechanism than does amphetamine.

Drug-induced stimuli Pentobarbital Amphetamine Fenfluramine Nicotine

FENFLURAMINE is an anorectic drug in humans that manifests some, but not all, of the pharmacological actions of amphetamine and related phenethylamines. Like these compounds, fenfluramine decreases food intake in animals [12] and is anorexigenic in man [8]. In behavioral tests, fenfluramine does not induce amphetamine-like stereotypy and it is generally reported to lack psychomotor stimulant actions in animals and man [29,30]. Indeed, fenfluramine has been reported to produce drowsiness in man [18]. In experiments using rats, fenfluramine has been successfully employed to control discriminative stimulus responding [7]. Thus, fenfluramine at a dose of 3.0 mg/kg was found to possess discriminative stimulus properties that controlled lever selection in a 2-lever operant task, and rats trained to discriminate the fenfluramine cue failed to generalize to the administration of 0.25–1 mg/kg amphetamine [7]. This lack of transference between these 2 anorectic drugs had previously been shown to occur in rats trained to discriminate amphetamine from saline which were unable to generalize to fenfluramine [26].

It has often been suggested that the smoking of tobacco products leads to a decrease in food intake [9] and the pharmacologically-active nicotine component is thought to mediate this effect [3]. In addition, nicotine has been reported to produce behavioral effects that resemble those produced by amphetamine [15,17]. Nicotine has been shown to possess discriminative properties in rats and tests of generalization of the nicotine stimulus cue to various doses of *d*-amphetamine indicates that rats do not perceive these two drugs as the same [24]. Furthermore, in rats trained to discriminate between *d*-amphetamine and saline, nicotine was unable to produce drug-appropriate responding [26].

The present study endeavored to investigate the degree to which rats trained to discriminate between *d*-amphetamine and pentobarbital would generalize to the effects of various doses of fenfluramine and nicotine by using the method of extended schedule perseverance [22]. It was expected that by training rats to discriminate between amphetamine (a stimulant agent) and pentobarbital (a sedating agent), the extent of the stimulant-like and depressant-like effects of fenfluramine and nicotine would be evidenced.

METHOD

Subjects

Twelve male ARS/Sprague-Dawley rats were individually housed with water ad lib under a 12 hr light-dark cycle. These experimentally-naive rats weighed 220–260 g at the start of the experimentation and were maintained at approximately 85% of their free-feeding weights as ascertained by daily weighing of an ad lib food control rat obtained from the supplier (Zivic-Miller, Allison Park, PA) at the same time. The rats were trained and/or tested at the same time of day, 5 days per week and each session was followed by an adjusted feeding of a commercial rat chow.

Apparatus

The experimental space was a standard rodent Skinner

test cage (Lafayette Instrument Co.) equipped with 2 levers placed 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 levers. The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and 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

The drug discrimination procedure employed has been described in detail elsewhere [23]. Six rats were administered 0.8 mg/kg *d*-amphetamine sulfate (as base) intraperitoneally (IP) and, 30 minutes later, were trained to press the left lever to receive a food reinforcement (45 mg Noyes pellet) under a fixed ratio (FR 1) schedule, whereas the other six rats were administered 6 mg/kg sodium pentobarbital (as base; IP), and 30 minutes later, were trained to press the same lever on the same FR 1 schedule. Training continued as the FR schedule was gradually increased until the rats were pressing the drug-appropriate lever on an FR 10 schedule. The number of consecutive daily sessions conducted to reach FR 10 responding was kept constant (13 sessions). Once the 2 groups of rats were observed to consistently press the first lever on the FR 10 schedule, they were injected with the other treatment (i.e., the amphetamine-trained rats received pentobarbital and the pentobarbital-trained rats received amphetamine) and they were required to press the opposite lever on the FR 1 schedule. Training continued in daily 15 min sessions until the second lever was pressed on an FR 10 schedule and the number of sessions to the second lever FR 10 criterion was kept constant (9 sessions) for all subjects.

Once consistent FR 10 responding was observed to occur on the second lever each group of rats received either 0.8 mg/kg *d*-amphetamine (A) or 6 mg/kg pentobarbital (P) on a 2-week alternating schedule: P-A-A-P-P; A-P-P-A-A. The lever first pressed 10 times was designated as the "selected lever" and training on the drug-appropriate lever continued for 15 min. Discrimination training continued until all rats were observed to select the appropriate lever first on a minimum of 9 of 10 training sessions.

Dose-Response and Drug Testing

During continued experimentation, 15 min maintenance sessions, with alternating administrations of 0.8 mg/kg *d*-amphetamine and 6 mg/kg pentobarbital, were continued on Mondays, Wednesdays, and Fridays to insure and maintain discrimination to the training conditions. On Tuesdays and Thursdays, each group of rats received either decreasing doses of pentobarbital and amphetamine or various doses of fenfluramine (1.13–2.25 mg/kg, as base) and nicotine (0.1–0.4 mg/kg, as base). Each dose was tested in a random order on 2 occasions with each test session preceded by both an amphetamine and a pentobarbital maintenance session. During these test sessions, a recently reported measurement [22], viz., extended schedule responding was employed. Thus, the rats were allowed to lever press, in extinction, until 10 responses were made on the lever that was *not* their first choice lever selection, e.g., if the rat was administered a dose of amphetamine less than the training dose of 0.8 mg/kg and it had pressed the "amphetamine-correct" lever 10 times that rat was allowed to continue pressing (without reinforcement) until 10 presses were made on the "pentobarbital-correct" lever. The number of lever presses made on the amphetamine-correct lever before 10 presses were accumulated on the pentobarbital-correct lever was recorded. Likewise, after administration of lowered pentobarbital doses (less than 6.0 mg/kg) the rats were allowed to press in extinction until 10 presses were accumulated on the amphetamine correct lever. After the administration of the various doses of fenfluramine or nicotine, the number of presses on each lever before 10 presses on the opposite lever were made was recorded and no reinforcement was given. Administration of all lowered doses of amphetamine and pentobarbital and all doses of fenfluramine and nicotine were made intraperitoneally without the experimenter (technician) knowing the substance administered, and all behavioral tests were conducted 30 min post-injection.

Statistics

Lever selections (first 10 responses) after all treatments are expressed as a percentage of rats observed to select the amphetamine-lever (AL). The number of presses made on the AL prior to 10 presses on the pentobarbital lever (PL) were compared to the number of presses accumulated on the PL prior to 10 presses on the AL in the same test trial by a paired *t*-test with $p<0.05$ chosen as the level of significance (two-tailed). Dose-response data were subjected to the method of Litchfield and Wilcoxon [14] which employs probit vs log dose plots and generates ED50's and tests of parallelism for best-fitted curves.

RESULTS

Rats trained to discriminate between *d*-amphetamine and pentobarbital by administering these drugs from the earliest stage of conditioning selected the appropriate lever on 9 of 10 training sessions after 2 two-week schedules of administration. The time period needed to acquire the two-drug discrimination training replicates a recent study [23] and confirms that this methodology is advantageous in training drug vs drug as it was in training drug vs vehicle [19]. The (0.8 mg/kg) training dose of amphetamine produced 100% responding on the AL and 0.4 mg/kg produced 91.6% amphetamine-appropriate lever selections. Both of these doses resulted in a significantly greater perserverance on the AL than on the PL, whereas the administration of 0.2 mg/kg amphetamine resulted in 58.3% AL selection and similar mean perseverances on both levers. Likewise, the training dose of pentobarbital produced 100% PL selection (0% AL) and significantly greater perseverance on the PL. When an unpaired *t*-test of means is applied to the AL response after the training dose of 0.8 mg/kg amphetamine (231.7 ± 135.9) and the PL responses after 6.0 mg/kg pentobarbital (132.3 ± 67.3) the means are shown not to be significantly different suggesting that the perception of the interoceptive cues produced by the training doses of the two drugs are approximately equivalent. Decreasing doses of pentobarbital resulted in a dose-dependent increase in percent AL selection (or a decrease in PL selection).

Figure 1 presents the dose-response curves for *d*-amphetamine and pentobarbital graphed according to the method of Litchfield and Wilcoxon [14] with the Y-axis being "percent drug-appropriate" first lever selections. The ED50's for amphetamine and pentobarbital generated by these curves are 0.19 mg/kg (95% confidence range: 0.14–0.27 mg/kg) and 1.45 mg/kg (1.05–2.00 mg/kg), respectively. These curves are parallel (S.R.=1.008; f.S.R.=1.01) within

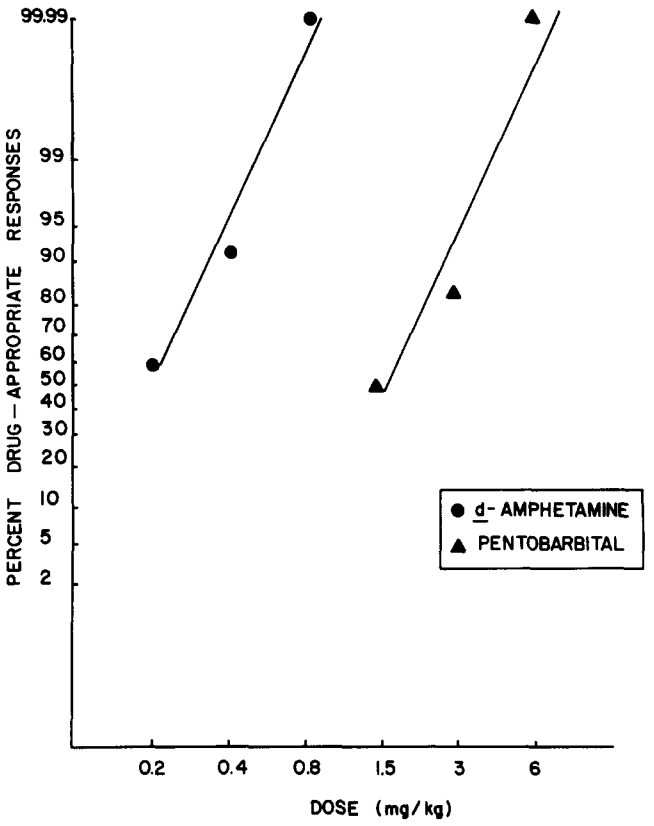

FIG. 1. Dose-response discriminative effects of (0.2–0.8 mg/kg) *d*-amphetamine and (1.5–6.0 mg/kg) pentobarbital. Ordinate: Percent of rats (n=12) selecting (responding 10 times first upon) drug-appropriate (correct) lever on probit scale. Abscissa: Log dose (in mg/kg) of *d*-amphetamine or pentobarbital.

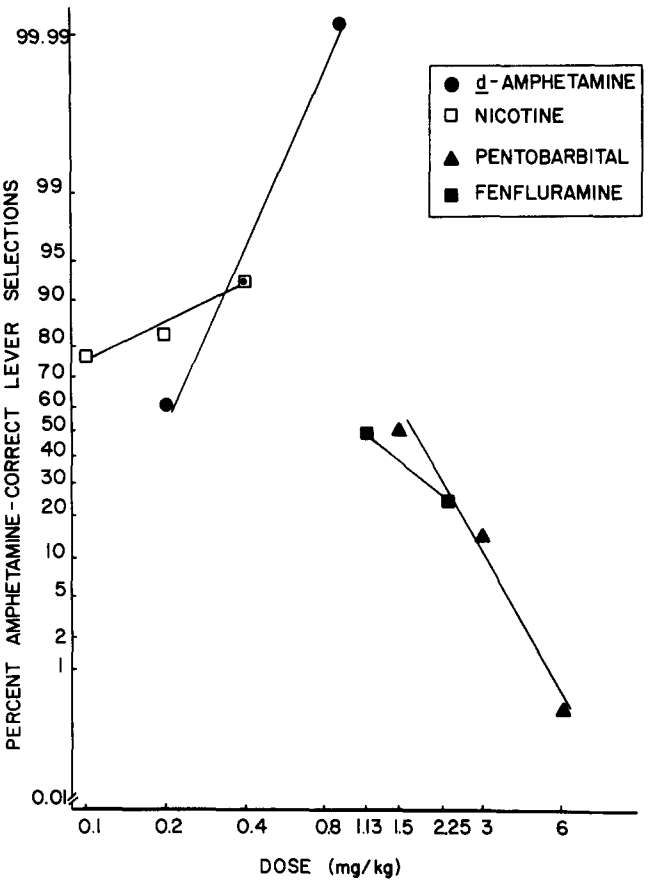

FIG. 2. Dose-response effects of nicotine and fenfluramine in rats trained to discriminate between 0.8 mg/kg *d*-amphetamine and 6 mg/kg pentobarbital. Ordinate: Percent of rats selecting amphetamine-correct lever on probit scale. Abscissa: Log dose of each drug.

statistic limits and the potency ratio is 7.63, i.e., *d*-amphetamine is 7.6 times as potent as pentobarbital and the drugs differ significantly in potency (f.P.R.=1.62).

Whereas pilot work showed that a 3.0 mg/kg dose of fenfluramine produced behavioral disruption in these rats, the 2.25 mg/kg dose produced 75% lever selection on the PL (25% on AL) and the lowest dose (1.13 mg/kg) produced 50% selection on the PL. All fenfluramine doses produced statistically non-significant differences in perseverance on each of the two levers. The highest dose of nicotine produced 91.7% AL selection and lower doses generated a dose-responsive decline in AL selection. All nicotine doses produced significantly ($p<0.05$) greater perseverance on the AL than on the PL. Figure 2 presents these results in graphical form with the Y-axis being the percent AL selections. Thus, increasing doses of amphetamine are seen to produce greater percentage of AL selections, whereas increasing pentobarbital doses are seen to produce decreasing AL selections, i.e., greater PL selections. The dose-response effect following the administration of the three nicotine doses is observed to generate a line that intercepts the *d*-amphetamine dose-response line and when the discriminative effects of the highest and lowest dose of fenfluramine are graphed, the best fitted [14] dose-response line intercepts the pentobarbital dose-response line.

DISCUSSION

The results indicate that imposing two drug states from the earliest stage of training in a two-lever food-motivated operant task produces rapid acquisition of a drug vs drug discrimination confirming a previous report [23]. In addition, extended schedule testing allows measurements of the perseverance of lever selection to each drug state and this procedure indicates the possibility of equivalent discriminable drug dosages [22]. Indeed, in the present study the perseverance on the amphetamine-lever after 0.8 mg/kg *d*-amphetamine and the perseverance on the pentobarbital-lever after 6.0 mg/kg pentobarbital were not significantly different suggesting that these two drug doses produced discriminable cues that were perceived equally.

The dose-response relationship of lowered doses of amphetamine and pentobarbital generated curves (lines) that were parallel with *d*-amphetamine being significantly more potent. In general, when dose-response curves of two drugs are parallel, it suggests that the drugs may be acting via a common site and/or mechanism of action [13]. In the case of amphetamine and pentobarbital, the simplest explanation for a common site of central action would be the reticular activating system since this brain area is thought to be responsible for wakefulness. Indeed, amphetamine can reverse the depressant effect of barbiturates on this brain area and it

lowers the threshold for arousal by electrical stimulation of this region [1].

Fenfluramine is a phenethylamine derivative which appears to have a pharmacological profile different from that of amphetamine [20] although some animal and human studies have indicated a similarity between these two anorectic agents [2, 21, 27]. When rats were trained to discriminate between fenfluramine and saline, 0.25–1.0 mg/kg *d*-amphetamine produced saline-like effects [7] and when rats were trained to discriminate between *d*-amphetamine and saline, 4–8 mg/kg fenfluramine produced saline responding [26]. In the present experimentation, in which rats were trained to discriminate *d*-amphetamine from pentobarbital rather than *d*-amphetamine from saline, fenfluramine again did not produce amphetamine-like discriminative responding.

Likewise, rats trained to discriminate nicotine from saline discriminate *d*-amphetamine as saline [24] and rats trained to discriminate between amphetamine and saline discriminate nicotine as saline [26]. In light of Overton's [19] suggestion that rats trained to discriminate one drug from another might be more sensitive to the discriminative properties of each than those rats trained to discriminate these drugs from saline, the present study indicated that when rats are trained to discriminate between amphetamine and pentobarbital, they perceive nicotine as of greater similarity to amphetamine than to pentobarbital.

Although fenfluramine produces pentobarbital-like responding and nicotine produces amphetamine-like responding, it appears that different sites and/or mechanisms of action are responsible for each of these similar effects. Since the nicotine dose-response curve intercepts the amphetamine dose-response curve (Fig. 2), it is suggested that these drugs act differently to produce their respective discriminative cues. However, Domino [4] reported that the electroencephalographic and behavioral arousal effects of nicotine may be mediated through the mesencephalic reticular formation. Previous work has indicated that the amphetamine cue is mediated by dopaminergic mechanisms [11] and that the nicotine cue is mediated by central cholinergic mechanisms [10,25]. Likewise, the interception of the fenfluramine and pentobarbital dose-response curves indicate different mechanism/sites of action. Various investigations indicate that fenfluramine acts via serotonergic mediation [5, 6, 28], whereas barbiturates have been evidenced to have a gamma-aminobutyric acid (GABA)-like action or to enhance the effects of GABA [16].

In summary, the results suggest that fenfluramine is perceived similar to pentobarbital in rats trained to discriminate between the central stimulant amphetamine and the central depressant pentobarbital but that fenfluramine acts differently than does pentobarbital. Likewise, nicotine is perceived more similar to amphetamine than to pentobarbital in these trained rats but it appears to act differently than does amphetamine.

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