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A Dose-Response Study of Anorectic Drug Effects on Food Intake, Self-Stimulation, and Stimulation-Escape[1]

CAROL L. KORNBLITH[2] AND BARTLEY G. HOEBEL

Department of Psychology, Princeton University, Princeton, NJ 08540

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KORNBLITH, C. L. AND B. G. HOEBEL. *A dose-response study of anorectic drug effects on food intake, self-stimulation, and stimulation-escape.* PHARMAC. BIOCHEM. BEHAV. 5(2) 215–218, 1976. A comparison was made of the short-term effects in rats of 3 anorectic drugs (amphetamine, fenfluramine, and phenylpropanolamine) on food intake and responses to obtain brain stimulation and to escape from automatic brain stimulation. At a dose which decreased food intake, amphetamine increased self-stimulation, but not stimulation-escape. Fenfluramine decreased both self-stimulation and stimulation-escape. Phenylpropanolamine, on the other hand, decreased self-stimulation, but not stimulation-escape. Even though all 3 drugs decreased food intake, each of them had different effects on hypothalamic self-stimulation and stimulation-escape. Only the actions of phenylpropanolamine were in agreement with the hypothesis that lateral hypothalamic reward and aversion reflect the animal's tendency to eat, suggesting that other aspects of reinforcement are also involved in lateral hypothalamic stimulation and were affected differently by these drugs.

Anorectic Food intake Self-stimulation Stimulation-escape Amphetamine Fenfluramine Phenylpropanolamine

A VARIETY of manipulations that influence feeding have been shown to have a similar influence on responding for electrical brain stimulation in the lateral hypothalamus [12]. It has been suggested that electrical stimulation of the lateral hypothalamus generates a combination of reward and aversion which shifts along a continuum depending on the animal's homeostatic balance [10,11]. According to this hypothesis, the reward of lateral hypothalamic stimulation is in part the specific reinforcing properties of food. When the animal's energy supplies are low, the animal eats readily and lateral hypothalamic stimulation is reinforcing; when there is an energy surplus, the animal refuses to eat and lateral hypothalamic stimulation is aversive. It has therefore been considered paradoxical that amphetamine decreases feeding and increases self-stimulation rates [16, 17, 18]. In the present study, the actions of amphetamine on food intake, self-stimulation, and stimulation-escape were compared to those of 2 other anorectic drugs, fenfluramine and phenylpropanolamine, in order to determine if there is some common behavioral action among them that might account for their appetite suppressing property. Dose-response curves were obtained for each drug on these behavioral measures. Compared to amphetamine, phenylpropanolamine is relatively non-stimulatory [4], and fenfluramine is a mild depressant [15,23]. It was expected that an anorectic drug which does not increase activity level might have a parallel action on feeding and self-stimulation and opposite action on stimulation-escape.

METHOD

Adult male Sherman rats, weighing from 250- 350 g, were individually housed.

Food Intake Test

Thirty rats were randomly distributed into 3 groups of 10 each. Animals were given at least 2 weeks to adjust to a 4 hr daily feeding schedule. Water was available at all times. Testing began when animals were consuming an amount of Purina pellets during the 4 hr period equivalent to the amount eaten during a 24 hr period with free access to food. Once per week animals were injected with one of the anorectic drugs, and 1 hr and 4 hr food intake were recorded. The range of doses selected for testing was intended to include one dose which decreased food intake to approximately 50% of control level, and one dose which had little or no effect on food intake. Animals in the first group were tested with fenfluramine hydrochloride in doses of 1.0, 5.0, and 10.0 mg/kg. In the second group, animals were tested with d-amphetamine sulphate in doses of 0.05, 0.01, and 1.0 mg/kg. Animals in the third group were tested with phenylpropanolamine hydrochloride in doses of 1.0,

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[2] Department of Psychology, University of North Carolina, Chapel Hill, North Carolina 27514.

5.0, and 10.0 mg/kg. All injections were made IP. Amphetamine and phenylpropanolamine were injected one half hr before the test. Fenfluramine was injected 1 hr before the test. Each animal was tested with all the doses for a given drug and with physiological saline. The order of testing the various doses was randomly determined. No animal was tested with more than one drug.

Brain Stimulation Test

The 17 rats used in this part of the experiment had free access to food and water in their home cages. Animals were anesthetized with 50 mg/kg sodium pentobarbital and implanted with monopolar, platinum-iridium electrodes, 0.23 mm in dia., insulated except for the conical tip. Electrodes were aimed at the lateral hypothalamus: 6 mm anterior to earbar zero; 1.75 mm lateral to the center of the mid-sagittal sinus; 7.5 mm below the dura with the skull horizontal between bregma and lambda. An indifferent electrode made contact with several skull screws. Tests were conducted in a Plexiglas box measuring 30.5 x 18 x 59 cm with a grid floor and open top through which the wire passed which connected the electrodes to the slip rings and stimulation apparatus. A response bar 2.5 x 1.9 cm was mounted 2.5 cm above the floor on each of the narrow walls. During self-stimulation (SS) tests, a response on one bar triggered a 0.5 sec train of 100 Hz, biphasic, 0.1 msec pulses. Additional presses within the 0.5 sec period did not affect stimulus duration and were not counted. For each animal a current level was determined which produced a moderate level of responding. Animals were also briefly screened for elicited feeding at this same current level. Food pellets were placed in the box and the animal was stimulated in a 30 sec on 60 sec off pattern for several cycles. If the animal ate during the stimulation in at least 2 out of 3 cycles and did not eat during the off periods, it was considered a stimulation-bound feeder. All but one of the animals met this criterion. Animals were then trained on stimulation-escape (SE). A response on the other bar turned off for 5 sec, stimulation delivered automatically in 0.5 sec trains, 1 sec apart. This test was used to detect changes in the negative reinforcement value of the stimulation and also served as a control for changes in general activity level. Animals were given 1 hr per day sessions of alternating 5 min SS and SE periods until their rates stabilized. During testing, 1 hr sessions (one half hr total SS and one half hr total SE) were run 5 days per week. On the fifth day animals were tested with one of the anorectic drugs. The doses, timing, and route of injection were the same as those used in food intake tests. Each animal was tested with all the doses for a given drug and with physiological saline. The order of testing the various doses was randomly determined. Results are expressed as percent of the average in the 4 no drug sessions preceding each drug test. No animal was tested in more than 2 of the drug series. Following completion of the experiment, animals were sacrificed with an overdose of anesthetic and perfused with 10% Formalin. The brains were sectioned at 50 μ, stained with Cresyl violet, and the electrode tips localized. The data for each group were analyzed by a one-way repeated measures analysis of variance and by subsequent Newman-Keuls comparisons [25].

RESULTS

There was at least one dose of each drug which significantly decreased 1 hr food intake. Fenfluramine was the only drug that had a significant effect on 4 hr food intake. There was no common behavioral effect of all 3 drugs on responding to obtain or to escape brain stimulation which might account for their appetite suppressing property.

Food Intake Test

As shown in Fig. 1, fenfluramine in doses of 5.0 and 10.0 mg/kg significantly decreased both 1 hr and 4 hr food intake, $F(3,28) = 16.7$, $p<0.01$, and $F(3,28) = 13.4$, $p<0.01$, respectively. Although different from control, subsequent comparisons indicated that the effects of these 2 doses did not differ significantly from each other. With amphetamine (see Fig. 1) only the 1.0 mg/kg dose significantly decreased intake, and this reduction occurred only in the 1 hr intake, $F(3,28) = 12.5$, $p<0.01$. As shown in Fig. 1, all doses of phenylpropanolamine significantly decreased 1 hr intake, $F(3,28) = 14.4$, $p<0.01$. Subsequent comparisons indicated that these doses did not differ from each other. With the doses used here, phenylpropanolamine did not significantly decrease 4 hr intake.

Brain Stimulation Test

As shown in Fig. 2, all doses of fenfluramine decreased SS rates, $F(3,28) = 33.0$, $p<0.01$. Subsequent comparisons indicated that the 5.0 and 10.0 mg/kg doses of fenfluramine differed significantly from the 1.0 mg/kg dose, although these doses did not differ from each other. Fenfluramine in doses of 5.0 and 10.0 mg/kg also decreased SE rates, $F(3,28) = 37.6$, $p<0.01$, although these doses did not differ from each other. During control no drug sessions, the average SS rate for the fenfluramine group was 560 responses per half hr and the average SE rate was 89 responses per half hr.

With amphetamine (see Fig. 2) the 1.0 mg/kg dose significantly increased SS rates, $F(3,18) = 7.6$, $p<0.01$, while none of the doses used had a consistent effect on SE rates, $F(3,18) = 1.4$, $p>0.01$. For the amphetamine group, the average SS rate during control no drug sessions was 709 responses per half hr and the average SE response rate was 58 responses per half hr.

As shown in Fig. 2, all doses of phenylpropanolamine significantly decreased SS rates, $F(3,28) = 18.9$, $p<0.01$. Subsequent comparisons indicated that the 5.0 mg/kg dose was significantly different from the 20.0 mg/kg dose, while the dose of 10.0 mg/kg did not differ from either the 5.0 or 20.0 mg/kg dose. Phenylpropanolamine in doses of 5.0 and 10.0 mg/kg increased SE rates slightly, overall $F(3,28) = .91$, $p>0.01$. The average SS rate for the phenylpropanolamine group during control no drug sessions was 718 responses per half hr and the average SE rate was 94 responses per half hr.

All but one of the brains were prepared histologically. All of these electrodes were localized anatomically in the dorsal portion of the medial forebrain bundle from the posterior portion of the anterior hypothalamic nucleus to the anterior tip of the ventromedial hypothalamic nucleus.

DISCUSSION

There was no common behavioral effect observed in all 3 anorectic drugs on the SS and SE tests in this study which could explain the appetite suppressing property of the

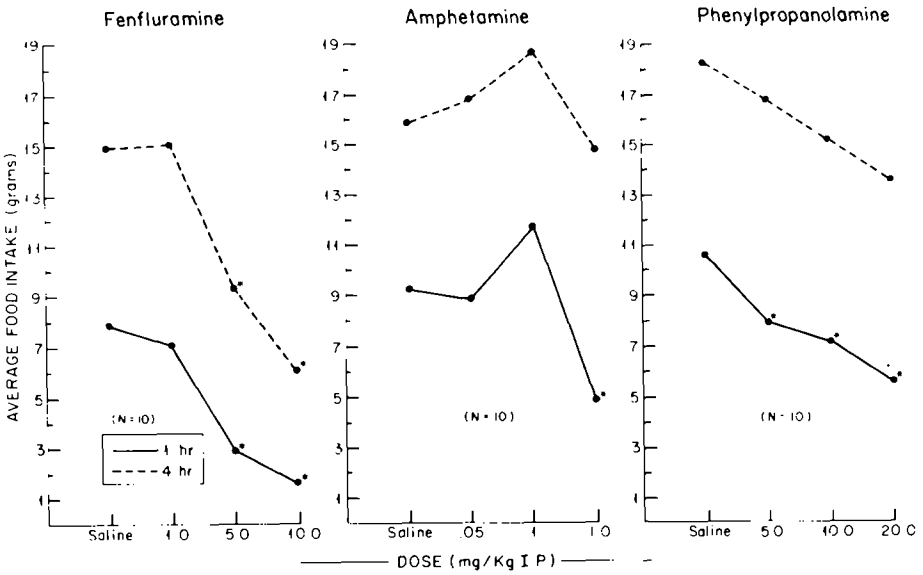

FIG. 1. Dose-response curves for the effects of anorectic drugs on average food intake during 1 hr and 4 hr periods with animals adapted to a 4 hr daily feeding schedule. Fenfluramine injected 1 hr before test. Amphetamine and phenylpropanolamine injected one half hr before test. *$p<0.01$

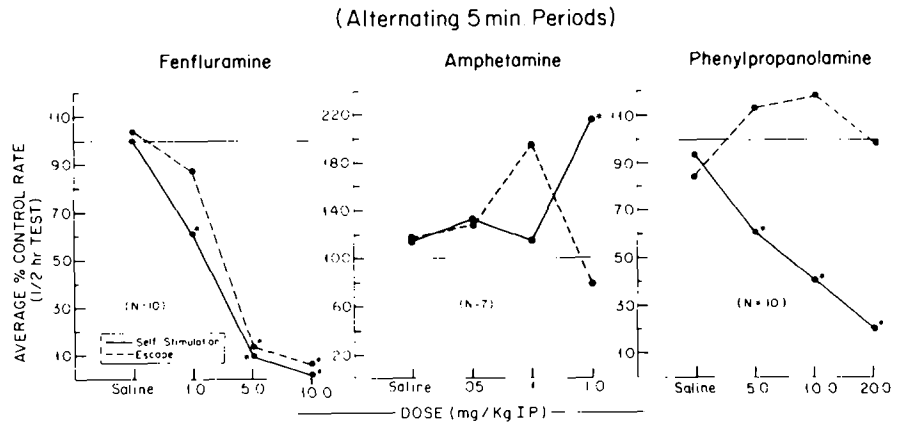

FIG. 2. Dose-response curves for the effects of anorectic drugs on self-stimulation and stimulation-escape expressed as average percent of control rate. Fenfluramine injected 1 hr before test. Amphetamine and phenylpropanolamine injected one half hr before test. *$p<0.01$

drugs. As expected, at an effective dose for decreasing food intake, fenfluramine decreased SS rates, but SE rates were also decreased. All of these effects may be due to the drug's general depressant effect on activity [15,23]. At the highest dose of fenfluramine, animals appeared sluggish.

Amphetamine increased responding in SS tests, but did not significantly affect responding to escape stimulation. If the increase in SS rates was due primarily to a general increase in activity then SE rates should have increased too. In this study, the enhancing effect of amphetamine on SS rates appeared to be due to its influence on the reward value of the lateral hypothalamic stimulation rather than to its influence on general activity.

Phenylpropanolamine decreased SS rates, while slightly increasing SE rates. At the highest dose, activity level did not appear to be affected, but the animals did display piloerection and urination. The decrease in SS rates can not be attributed to a decrease in general activity level because SE rates were not similarly decreased. This decrease in SS rates may reflect a decrease in the reinforcement value of electrical stimulation of the lateral hypothalamus in a non-hungry animal. Data from other SE tests using higher doses and longer durations indicate that phenylpropanolamine can significantly increase SE responding [13].

It is not clear from this study why amphetamine increases self-stimulation rates, whereas the other 2 drugs decrease it. We presume that amphetamine activates some lateral hypothalamic reward system unrelated to food reward. Thus the hypothesis that the reward from stimulation of the lateral hypothalamus is like food reward is an

oversimplification. It accounts for the effects noted with phenylpropanolamine, but not for those of amphetamine and fenfluramine.

Although the mechanism of action of these anorectics is not fully understood, presumably the drugs are causing metabolic changes and also acting on different neurotransmitter systems. Amphetamine may influence catecholamine neurons by facilitating release of both dopamine and norepinephrine, by blocking their reuptake, or by inhibiting the action of monoamine oxidase [3,24]. Amphetamine may activate catecholaminergic satiety functions as shown by the fact that norepinephrine [1] or dopamine [9] depletion decrease amphetamine anorexia. The increase in SS rates observed with amphetamine supports the idea of a catecholaminergic substrate for lateral hypothalamic SS [7, 18, 22].

Much less is known about phenylpropanolamine. It is a sympathomimetic similar in properties and potency to ephedrine, but it has less central nervous system activity. Ephedrine is thought to release norepinephrine and also to have a direct effect on both α and β receptors. It is less active centrally than amphetamine [8]. Phenylpropanolamine is like amphetamine and fenfluramine in that all 3 drugs decrease food intake in rats with ventromedial hypothalamic lesions [2,4]. With phenylpropanolamine there was no noticeable change in activity level [4].

Recent investigations of fenfluramine indicate that its anorectic effect may be due to an action on serotonergic neurons [6, 14, 21]. When forebrain serotonin was lowered by lesions of the midbrain raphe, the anorectic effect of fenfluramine was antagonized; the same lesion had no effect on amphetamine anorexia [20]. On the other hand, when catecholamines were depleted the anorectic effect of amphetamine was antagonized, but the same depletion potentiated the action of fenfluramine [5].

Therefore these anorectic drugs are acting on at least two, possibly more, different neurotransmitter systems which pass through the lateral hypothalamus. These drugs have similar effects on food intake, although these effects appear to be mediated by different behavioral systems. None of the drugs produce the normal behavioral signs of satiety such as grooming followed by sleep [19]. These drugs differ in their influence on responding to obtain or to escape electrical stimulation of the lateral hypothalamus. The effects of amphetamine and fenfluramine on SS and SE rates appear to be independent of anorexia. There may be a difference between hypothalamic reinforcement related to feeding and that related to nonspecific operant behavior or activity level. The effects of phenylpropanolamine on lateral hypothalamic SS and SE rates appeared to be consistent with its effects on feeding.

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