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The Appetite Suppressant, d-Fenfluramine, Decreases Self-Stimulation at a Feeding Site in the Lateral Hypothalamus

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McCLELLAND, R. C., T. SARFATY, L. HERNANDEZ AND B. G. HOEBEL. *The appetite suppressant, d-fenfluramine, decreases self-stimulation at a feeding site in the lateral hypothalamus.* PHARMACOL BIOCHEM BEHAV 32(2) 411–414, 1989.—In prior studies rats showed a relative shift from self-stimulation to escape (i.e., from reward to aversion) following a large meal, obesity or anorectic doses of insulin. Racemic fenfluramine, on the other hand, decreased both self-stimulation and escape suggesting it had a general behavior suppressant property. To avoid the depressive, antidopaminergic effects of the l-isomer, this study tested the d-isomer which is primarily serotonergic. Rats were screened for stimulation-induced feeding and then trained to self-stimulate with one lever in 5-min periods that alternated with 5-min periods of automatic stimulation from which the animal could escape with a different lever. d-Fenfluramine (1.5–4.5 mg/kg IP) caused a dose-related decrease in self-stimulation. Stimulation-escape was relatively unaffected. This is interpreted as a decrease in feeding reward due to d-fenfluramine.

Self-stimulation Reward d-Fenfluramine Hypothalamus Rat

STIMULATION through some lateral hypothalamic (LH) electrodes induces eating. Perhaps this is because it mimics the effects of palatable tastes on neuronal firing of hindbrain taste neurons (17), and because it releases dopamine in the mesolimbic system (6). The stimulation also reinforces operant behavior as shown by self-stimulation. The rate of self-stimulation can vary with the animal's food intake and body weight (10). This suggests that the reward of self-stimulation at the LH site is modulated by physiological systems which control feeding (5) and that self-stimulation can mimic the reward of food (10).

When self-stimulation slows down after a meal, a control for lethargy is necessary (16). We find that after a meal rats work harder to escape from automatic stimulation (5). Stimulation-escape can also increase if the animal is made obese (11,13). Anorectic doses of insulin had the same effect; self-stimulation decreased and stimulation-escape increased (5). This shift from reward to aversion was also obtained with the anorectic drug, phenylpropanolamine (9,14). As a further control, the reward-aversion shift occurred selectively at LH electrodes without affecting a different self-stimulation site in the posterior hypothalamus of the same rats (5). Therefore, LH electrodes of the type which induce feeding, self-stimulation and stimulation-escape provide a way of tapping into a neural mechanism involved in the control of appetite.

Racemic, d,l-fenfluramine is an appetite suppressant which gave results that did not corroborate the above theory. Racemic fenfluramine decreased self-stimulation, but paradoxically stimulation-escape decreased as well (14). It is unlikely that the drug would decrease both reward and aversion at the same time unless it was a general behavior depressant. Racemic fenfluramine has long been recognized as an anorectic drug which decreases food intake without having the stimulant side effects of most other amphetamine analogues, and it is a mild depressant (8, 18, 26). Recently, the anorectic property of fenfluramine was found in the d-isomer and the depressant side effect was attributed to the l-isomer (4, 21, 22). Therefore, the l-isomer is probably responsible for the overall decrease in operant responding. The question for the present experiment was whether d-fenfluramine would suppress self-stimulation rate without depressing stimulation-escape responses.

METHOD

Eight female Sprague-Dawley rats weighing 300–360 g and one adult male Sprague-Dawley weighing 500 g were

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individually housed with free access to food and water. Monopolar, platinum-10% iridium electrodes, 0.23 mm in diameter, insulated except for the conical tip, were implanted in the perifornical region of the lateral hypothalamus: 6.5 mm anterior to the intraaural zero, 1.6 mm lateral to the midsagittal sinus, 8.5 mm perpendicularly below the skull surface.

The reward-aversion test was adapted from the design by Olds and Olds (19). Tests were conducted in clear plastic boxes measuring 30.5×18.0 cm with a grid floor and an open top through which the stimulating wires passed by way of a mercury commutator and counterbalance arm. There were two levers (2.5×1.9 cm) on opposite ends of the cage 2.5 cm above the floor. The self-stimulation lever triggered a 0.5 sec, 100 Hz train of 0.1 msec pulses passed through a transformer for zero net current flow. During alternate 5-minute periods, a 0.5-sec train of pulses repeated automatically once per second. The escape lever turned off this stimulation for 5 sec. Escape responding was used to detect changes in negative reinforcement as well as providing a control for basic motor capability.

The rats were first tested for stimulation-bound feeding with a continuous pulse train of 15–20 sec. Only those animals that started eating within 10 sec and continued eating for 10 sec were used. They were trained to self-stimulate and then left overnight with automatic stimulation in alternate 5-min periods to learn stimulation-escape. Only rats that were stimulation-bound feeders and that could consistently self-stimulate and escape were used in the experiment. Thirty rats were initially screened; nine met the above criteria. Most of the rats rejected were not stimulation-bound feeders; others never learned the escape response.

The baseline consisted of self-stimulation and stimulation-escape alternating every 5 min for 30 min. This was followed by an IP injection of either saline or d-fenfluramine (1.5, 2.5, 3.5, or 4.5 mg/kg) and another 90 minutes of self-stimulation and stimulation-escape to determine the effect of the drug. Five animals received two days testing with saline injections between each day with a drug injection. A second group of four animals received saline on just one day between each drug test.

Each animal was tested with each of the 4 doses in a predetermined manner. In the series: 4.5, 1.5, 2.5, 3.5, 4.5, 1.5 mg/kg, the first 2 rats of each group started at 1.5 and 2.5 mg/kg respectively with succeeding doses to the right. The other 2 rats of each group started at 3.5 and 4.5 mg/kg respectively in the series and went to the left. The last rat received 1.5 to 4.5 mg/kg in ascending order. The rate of self-stimulation and stimulation-escape was recorded by computer and cumulative recorder every 5 min and averaged in 30-min segments for calculation of percentage change from the preinjection baseline. Statistics were performed by analysis of variance followed, when warranted, by a Newman-Keuls test.

Animals were sacrificed with pentobarbital; brains were perfused with formalin, sectioned, and the electrode tips localized.

RESULTS

As shown in Figs. 1 and 2, all four doses of d-fenfluramine significantly decreased self-stimulation [$F(4,32)=39.6$, $p<0.001$; post hoc Neuman-Keuls $p<0.01$ for each dose], while causing no significant decrease in stimulation-escape. No residual effect of the drug was observed on self-stimulation baseline rates on saline days. At the highest dose

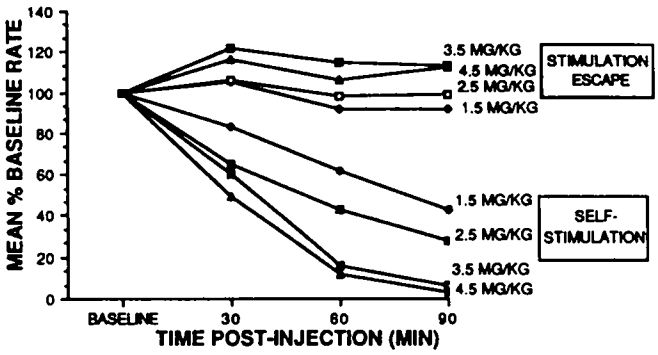

FIG. 1. d-Fenfluramine (IP) at the indicated doses decreased self-stimulation rate (lower curves), but not stimulation-escape rate (upper curves) in the same rats. The 30-min baseline is defined as 100% for comparison with 30-min periods postinjection. Saline control data (shown in Fig. 2) varied less than 10% from baseline for self-stimulation and escape.

(4.5 mg/kg), some animals appeared lethargic and some displayed piloerection and urination during stimulation-escape. Thus, the highest dose was unnecessarily high. This may have prevented stimulation-escape from increasing. Stimulation-escape did increase in some animals at some doses, but the mean effect on stimulation-escape was no change at any dose. The mean of mean self-stimulation rate before drug injection was 240 responses/5 min (range 197–278) and mean of mean baseline stimulation-escape rate was 11 responses/5 min (range 7–15). The principal effect was a 40% decrease in self-stimulation at the lowest dose tested (1.5 mg/kg).

Photomicrography of brain sections in three rats showed electrodes were adjacent to the fornix in the lateral hypothalamus.

DISCUSSION

Each of the four doses decreased self-stimulation while causing no significant change in stimulation-escape relative to saline tests or baseline tests. Self-stimulation rates started to decrease within 30 min after d-fenfluramine injection. This is not attributed to hypokinesia because stimulation-escape rate, which was relatively slow to start with, did not decrease (Fig. 1).

Comparing these results with our earlier studies, d-fenfluramine decreased self-stimulation like insulin (5,10) or the over-the-counter anorectic, d,l-phenylpropanolamine (PPA), and did not display the depressive or sedative property of racemic d,l-fenfluramine (14). d-Fenfluramine did not increase mean stimulation-escape in this test, which suggests it may have a different spectrum of neurochemical effects than PPA and may therefore suppress appetite in a different manner. The baseline rates of self-stimulation and stimulation-escape were not matched which could in theory introduce rate-dependent effects into the result. However, this is an unlikely explanation of the result because 3.5 mg/kg fenfluramine reduced rapid self-stimulation by 90% to about the same slow rate as escape; while the escape rate increased 10%. Thus, d-fenfluramine reduced the animals' efforts to turn stimulation on, without reducing efforts to turn it off.

Fenfluramine exerts part of its action in the hypothalamus. When injected directly in the lateral hypothalamus

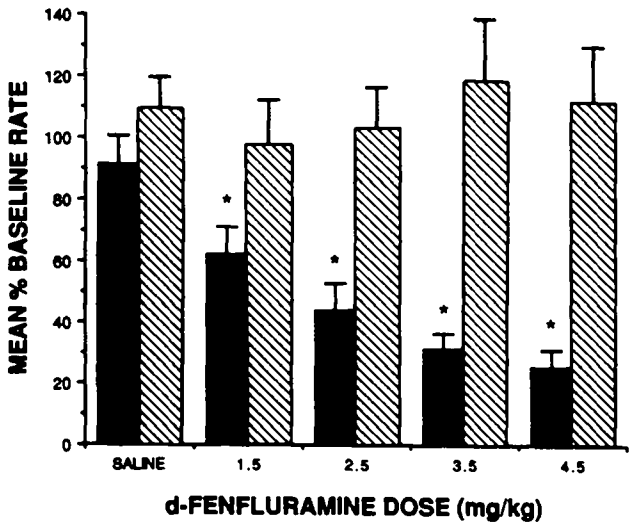

FIG. 2. The effect of d-fenfluramine at the indicated doses on 5-min periods of self-stimulation alternating with 5-min periods of stimulation-escape averaged over the course of the entire 90-min postinjection period (asterisks indicate $p<0.01$). Solid bars: self-stimulation; shaded bars: stimulation-escape.

(2,25) or paraventricular nucleus (12,15) it is effective in decreasing food intake in rats. Predictably, electrolytic lesions of the PVN reduce the anorectic effects of d-fenfluramine (3, 7, 8, 12). But for reasons that are not clear, lateral hypothalamic lesions (3,14) or depletion of the ventral noradrenergic bundle (1,7) enhance d,l-fenfluramine anorexia while diminishing amphetamine anorexia. In the intact animal, fenfluramine probably acts in part by inhibiting an LH feeding system and enhancing PVN-MH satiety system (12).

Fenfluramine has several neurochemical actions depending on the dose and site. It may bind to some of the same receptors as amphetamine (20). At high doses (12 mg/kg) it can deplete serotonin (23), although 0.5 mg/kg is effective in suppressing carbohydrate intake (Leibowitz, personal communication). Recent microdialysis studies in this laboratory show that d-fenfluramine can cause an increase in extracellular serotonin and dopamine turnover in the lateral hypothalamus of freely moving rats (24,25). This agrees with earlier pharmacological studies which show that d-fenfluramine is a serotonergic compound (4, 18, 21, 26).

Both catecholaminergic and serotonergic anorectic drugs may exert part of their appetite suppressant effect by inhibiting the LH reward system that is normally excited by food. These self-stimulation studies do not tell us which component of reward is inhibited. It could be a mechanism for foraging and self-administration, or for taste, or metabolic control, or all three. The present results suggest that d-fenfluramine at the lower doses can inhibit the LH feeding-reward system without decreasing stimulation-escape which was a sign of motor impairment or depression seen with racemic d,l-fenfluramine. In sum, LH stimulation can induce feeding and a reward that is inhibited by d-fenfluramine.

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