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Effects of Selective Forebrain Depletions of Norepinephrine and Serotonin on the Activity and Food Intake Effects of Amphetamine and Fenfluramine

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CAREY, R. J. *Effects of selective forebrain depletions of norepinephrine and serotonin on the activity and food intake effects of amphetamine and fenfluramine.* PHARMAC. BIOCHEM. BEHAV. 5(5) 519–523, 1976. – Selective forebrain depletions of either norepinephrine or serotonin were produced in separate groups of rats by placement of lesions in the brainstem noradrenergic area and in the dorsal and median raphé nuclei respectively. Rats with norepinephrine depleting lesions exhibited an attenuation relative to intact animals of both the anorexic and locomotor stimulatory effects of amphetamine. In contrast, depletion of serotonin by the raphé lesion enhanced the locomotor stimulation induced by amphetamine but did not affect the anorexic efficacy of amphetamine. Neither brain lesion, however, reliably altered the animals' response to either the anorexic or activity effects of fenfluramine.

Amphetamine Fenfluramine Activity Anorexia Brainstem lesions

EVER since the initial observation [6] that lesions placed in the medial hypothalamus produce voracious eating and obesity, the concept of a CNS satiety mechanism has been incorporated into theories of the regulation of food intake. Recently, a neurochemical dimension to the satiety mechanism has been suggested which implicates noradrenergic and serotonergic containing fiber systems in the mediation of satiety signals. One virtue of this proposal is that it accounts for the anorexic activity of both fenfluramine, a serotonergic drug, and amphetamine, a catecholaminergic drug. Consistent with this proposition, lesions placed in the noradrenergic system were shown to attenuate amphetamine induced anorexia [1] and lesions placed in the serotonergic midline raphé system blocked fenfluramine anorexia [13]. A considerable amount of conflicting data exists, however, particularly with regard to the effects of serotonin depletion on food intake and fenfluramine anorexia. Specifically, a number of studies of food intake in animals sustaining serotonin depletion produced either by raphé lesions or systemic drug treatment generally have not reported overeating and obesity, [8, 9, 11, 13, 14]. On the other hand, intracerebral injection of drugs which deplete serotonin can produce an exaggerated food intake [3,12]. More difficult is a recent report [13] which failed to observe a diminution of the anorexic efficacy of fenfluramine in animals with raphé lesions. In view of these experimental inconsistencies the present study was undertaken to compare directly the effects of norepinephrine and serotonin depleting lesions on the development of obesity as well as on the anorexic activity of amphetamine and fenfluramine.

METHOD

Animals

Twenty naive male, Sprague Dawley rats, 120 days old at the start of experimentation were used. The rats were maintained in a room with controlled temperature (70° ± 2° F) humidity (55% ± 5%) and light (12 hr light, 12 hr dark). Tap water and a high fat diet, 33% by weight Crisco brand vegetable fat, (Proctor and Gamble Co., Cincinnati, Ohio) and 67% Purina laboratory chow powder were provided. The high fat diet was used since it had been demonstrated [4] that this diet is much more effective than conventional diets in eliciting obereating and obesity in hypothalamic lesioned rats. In view of previous failures to observe obesity in rats with raphé lesions, the use of the high fat diet in the present study was designed to better optimize conditions for eliciting overeating.

Procedure

Initially, three surgical groups of 6 each were formed. There were 2 lesion groups and one sham operated group. In one lesion group the lesion was positioned to destroy brainstem noradrenergic cell bodies and for the other lesion

group the electrode was oriented to destroy the dorsal and median raphé nuclei. Since two animals in the noradrenergic group died a few days postoperative, a total of eight rats received this lesion in order to achieve 6 rats per group. The brain lesions were made with a temperature controlled radio-frequency lesion maker (Radionics, Inc). A Koph sterotaxic instrument was used to position the thermister containing electrode at the appropriate brain site. The stereotaxic coordinates were: Raphé lesion – 6.8 mm posterior to bregma, 7.1 and 8.0 mm below dura, 1.2 and 1.4 mm lateral to the midline, with the electrode angled 10° toward the midline. Norepinephrine lesion (NE) 6.2 mm posterior to bregma, 7.0 mm below dura, 1.4 mm lateral to the midline. The lesion coordinates were similar to those previously used by [10] which were shown to be effective in depleting forebrain norepinephrine and serotonin. The incisor bar was fixed at 3.2 mm above the interaural line. After the electrode was positioned within the brain, current was increased slowly until a temperature of 55° C was reached which was maintained for 60 sec. The surgery was aseptic and performed with the rats under deep ether anesthesia. After the operation, each animal was given an intramuscular injection of 200,000 units of procaine penicillin.

At the conclusion of the experiment all animals were decapitated and the brains rapidly removed. The brains were coronally cut at the crossing of the optic chiasm as was previously reported [10]. The rostral portion of the brain was used for norepinephrine, dopamine, and 5-hydroxytryptamine determinations. The caudal portion of brain tissue was placed in 10% Formalin for a 5 day fixation period. After this fixation period, 3 mm thick sections containing the lesions were embedded in paraffin. Subsequently, 6 μ thick sections were cut, mounted and stained with cresyl violet. All sections were examined microscopically to assess the lesions.

The brain tissue samples used for biochemical analysis were weighed to the nearest 0.1 mg and homogenized in 3.0 ml of ice cold 0.4 M percholoric acid. Tissue homogenates were centrifuged at a force of 20,000 g for 10 min at 4° C and supernatant was decanted and stored for 1 day at -20° C. These tissue extracts were assayed for norepinephrine, dopamine and 5-hydroxytryptamine content according to the procedure of [15]. Sample fluorescence was measured in an Aminco-Bowman spectrophotofluorometer equipped with a Xenon lamp. L-Arterenol bitartrate, dopamine HCl and 5-hydroxytryptamine (creatinine sulfate complex) (Sigma Chimical Co.) were used as standards. Tissue monoamine content was calculted as μg monoamine base per gram of brain tissue. Estimated recovery was 100%.

Testing Procedure. All animals were maintained on ad lib feeding prior to and for two weeks after surgery. After the two week postoperative recovery period the amphetamine and fenfluramine drug testing sequence was begun. There were three phases of testing: In the first phase the effect of each drug on food intake was tested, the second phase tested locomotor activity and the third phase was a retest of food intake. After the food intake retest there was a two week period of ad lib feeding and then the experiment was terminated.

Intake testing. The food intake testing was assessed in a one hour intake period following twenty-four hours of food deprivation. The twenty-four hour food deprivation procedure involved removing all food from each animal but keeping water ad lib. At the end of this food deprivation period each animal was injected with either saline or drug and 15 min later permitted food and water access for one hour. After the one hour intake period all animals were returned to ad lib feeding for twenty-four hours and then again put on food deprivation. Thus, there was a daily alternation of deprivation and ad lib feeding. There were a total of eight deprivations followed by one hour food intakes in both phases of intake testing. The first, third, fifth and seventh one hour intakes were preceded by saline injections and the other intakes were preceded by drug injections. The four drug injections were 0.5 and 1.0 mg/kg of d-amphetamine HCl (K and K Chemicals, Jamaica, N.Y.) and 1.0 and 2.0 mg/kg fenfluramine HCl (A. H. Robins Co., Roanoke, Va.). The drugs were given in descending order in terms of dosage and the drug order was counterbalanced at each dose level within each experimental group. The first intake testing was conducted between the third thru fifth weeks postoperative and the retest took place between the 9th thru 11th weeks postoperative. The food intake retest was the same as the first test except the drug dosage was in ascending order. All food and water intakes were measured to the nearest 0.1 g and spillage losses were collected and also measured. All testing was done in the animals' home cage using the high fat diet. All injections were intraperitoneal. Drug doses were calculated as the salt and were equal in volume, 1 ml/kg, and dissolved in 0.9% sodium chloride.

Activity testing. Two large photoactivity cages LVE No. 1497 (Lehigh Valley Electronics Co.) were used to assess drug-induced locomotor activity changes. These cages were cylindrical, 61.0 cm in diameter and 53 cm high, and had a wire mesh floor. Six infrared photocells placed 2.5 cm above the floor detected movements. Photobeam interruptions were recorded on two digital counters, with each counter recording from three photocells.

Since activity generally is highest on the first exposure to an activity cage, drug testing was not begun until animals had had three successive daily nondrug activity tests. The activity tests lasted 30 min per day with photobeam interruptions recorded every 10 min. There were 2 drug test days per week with at least 2 days separating drug tests. On each day before a drug test animals were given an activity test with the saline injection. The 0.5 and 1.0 mg/kg d-amphetamine and 1.0 and 2.0 mg/kg fenfluramine injections were given 15 min before the activity session and in ascending order in terms of dosage and the drug order was counterbalanced at each dose level within each experimental group. On the third week of activity testing the tests were repeated for the 1.0 mg/kg amphetamine and 2.0 mg/kg fenfluramine injections.

RESULTS

A simple comparison of the preoperative body weight to the body weights recorded at the end of the experiment was sufficient to demonstrate that only the norepinephrine (NE) lesion significantly enhanced body weight. As shown in Table 1 the body weight gains of the control and raphé groups were virtually identical but yet were less than 50% of the weight gained by the NE lesion group. This increase in body weight by the NE lesion group was significantly different statistically $p<0.01$ from both the control and raphé groups.

Table 2 summarizes the effects of amphetamine and

TABLE 1

MEANS AND STANDARD ERRORS OF BODY WEIGHT MEASURED PREOPERATIVELY AND AFTER 13 WEEKS POSTOPERATIVE

Group	N	Body Weight in Grams Preoperative	Final Postoperative
NE	6	480.0 ± 18.6	880.6 ± 32.0
Raphé	6	508.4 ± 8.3	681.2 ± 17.3
Control	6	490.0 ± 30.4	641.8 ± 33.0

fenfluramine on food intake. In both the initial testing and in the retest the intake of the NE lesion group was least affected by amphetamine. In the first testing sequence the N. E. group consumed significantly more food than the control and raphé groups at the 0.5 mg/kg dose ($p<0.01$) and the 1.0 mg/kg dose ($p<0.05$). On the retest the NE group consumed significantly more food only at the 0.5 mg/kg dose ($p<0.05$). In considering this suggestion of an increased sensitivity to amphetamine by the NE group on the food intake retest, it should be noted that between the first and second food intake tests the NE group had a much larger weight gain than the other two groups with a mean increment of 305 g for the NE group compared with mean increments of 190 and 134 g for the raphé and control groups respectively. Also important in Table 2 is the finding that the intakes of the control and raphé groups were decreased from the saline level by virtually the same amount for both dose levels of amphetamine on both testing sequences. With regard to fenfluramine, the result was similar in that the food intakes of the control and raphé groups were decreased by essentially the same amount for both dose levels of fenfluramine on both testing sequences. The intake results for the NE group under fenfluramine, however, are also important to consider. The decrease in intake for this group were always more than the controls and on the retest at the 2.0 mg/kg dose level this decrease reached the $p<0.05$ level of statistical significance.

On the activity measure the two lesion groups responded quite differently to amphetamine. The activity results summarized in Table 3 show that the raphé group exhibited a greater increase in activity following amphetamine than the controls. The greater increase of the raphé group was statistically significant $p<0.05$ for all amphetamine tests. In contrast to the raphé group, the NE lesion group exhibited an attenuated activity increase to amphetamine which was statistically significant at the $p<0.05$ level for the 1.0 mg/kg dose. The NE group, however, had a statistically significant lower baseline activity than the control group $p<0.05$. Fenfluramine consistently reduced activity in all

TABLE 2

MEANS AND STANDARD ERRORS FOR 1 HOUR FOOD INTAKES IN GRAMS OF OPERATED AND CONTROL GROUPS FOR AMPHETAMINE AND FENFLURAMINE TREATMENTS AND THE PRECEDING SALINE TEST DAYS

Group	N	Food Intake in Grams Saline	Amphetamine 0.5 mg/kg	Amphetamine 1.0 mg/kg	Fenfluramine 1.0 mg/kg	Fenfluramine 2.0 mg/kg
Test 1						
NE	6	8.2 ± 1.1	9.5 ± .5	7.0 ± 2.2	5.7 ± 1.0	2.3 ± 0.9
Raphé	6	6.1 ± 1.8	4.7 ± .8	0.8 ± .1	4.2 ± 1.3	0.8 ± 0.5
Control	6	7.6 ± 1.0	4.3 ± .6	1.9 ± .6	6.0 ± .9	2.7 ± 0.9
Test 2						
NE	6	8.3 ± 1.0	7.2 ± 1.2	.5 ± .4	4.9 ± .9	2.1 ± 1.0
Raphé	6	8.0 ± .6	3.6 ± 1.0	.6 ± .3	6.3 ± .5	3.5 ± 1.0
Control	6	7.1 ± .1	3.7 ± .5	1.1 ± .6	6.9 ± .6	4.9 ± 0.7

TABLE 3

ACTIVITY LEVELS EXPRESSED AS MEANS AND STANDARD ERRORS OF PHOTOBEAM INTERRUPTIONS RECORDED UNDER SALINE, AMPHETAMININE AND FENFLURAMINE TREATMENTS FOR THE OPERATED AND CONTROL GROUPS

Group	N	ACTIVITY Saline	Amphetamine 0.5 mg/kg	Amphetamine 1.0 mg/kg	Fenfluramine 1.0 mg/kg	Fenfluramine 2.0 mg/kg
Test 1						
NE	6	284.7 ± 42.1	312.6 ± 52.6	368.5 ± 41.1	134.8 ± 24.8	152.8 ± 20.9
Raphé	6	552.0 ± 122.4	1268.2 ± 54.1	1460.2 ± 265.1	425.7 ± 89.4	257.5 ± 62.4
Control	6	519.4 ± 76.4	625.1 ± 179.0	829.0 ± 116.8	305.5 ± 42.5	288.0 ± 59.3
Test 2						
NE	6	304.8 ± 44.9		322.3 ± 54.4		166.3 ± 25.8
Raphé	6	427.7 ± 54.2		1526.0 ± 185.6		337.7 ± 106.7
Control	6	470.8 ± 103.2		921.5 ± 186.1		266.3 ± 62.2

tests and at all dose levels used. Furthermore, this effect of fenfluramine was consistent for all groups.

Histological examination of the brain lesions revealed a discrete midline area of cavitation for the raphé nuclei in all but one animal. The NE lesions were predominantly bilaterally symmetrical and situated lateral to the ventral aspect of the central grey and well located to interrupt the ventral noradrenergic bundle and dorsal noradrenergic fibers as well. Generally, the lesions were comparable to those previously reported [10] which used similar stereotaxic coordinates In agreement with the histological picture the biochemical measurements shown in Table 4 indicate that the raphé lesions markedly and selectively depleted the forebrain section of 5-hydroxytryptamine and that the NE lesions selectively lowered the forebrain section norepinephrine levels. The lower levels of 5-hydroxytryptamine for the raphé group and the lowered norepinephrine levels for the NE lesion group were both statistically significant at the $p<0.01$ level.

TABLE 4

MEANS AND STANDARD ERRORS OF FOREBRAIN SECTION CONTENT OF NOREPINEPHRINE, DOPAMINE AND 5-HYDROXYTRYPTAMINE

Group	N	Norepinephrine μ g/g	Dopamine μ g/g	5-Hydroxytryptamine μ g/g
NE	6	0.15 ± 0.01	2.54 ± 0.03	0.56 ± 0.08
Raphé	6	0.389 ± 0.01	2.63 ± 0.18	0.089 ± 0.02
Control	6	0.391 ± 0.01	2.62 ± 0.14	0.59 ± 0.07

DISCUSSION

The occurrence of obesity and an attenuated anorexic response to amphetamine in animals sustaining lesions of the brainstem noradrenergic area is in accord with previous reports [1,2]. One interpretation of this attenuated amphetamine response might be that it is more difficult to inhibit feeding in animals having lesions which induce excessive food intake. Such a general non-specific interpretation, however, appears unlikely in view of the additional finding in the present study that the anorexic effect of fenfluramine tended to be enhanced in these noradrenergic lesioned animals. Thus, the present study strengthens the argument that a norepinephrine system is one component of the food intake satiety mechanism as well as being a mediator of the anorexic response in amphetamine. The complementary finding that norepinephrine lesions also attenuated the locomotor stimulatory effect of amphetamine is consistent with a noradrenergic involvement in the mediation of at least the low dose level effects of amphetamine.

The present experiment, in agreement with several previous reports [8,14] failed to support the proposition that lesions of serotonergic systems impair the satiety mechanism or are necessary for the anorexic activity of fenfluramine. Studies are needed, however to ascertain the brain for the discrepancy between the failure of raphé lesions to induce overeating but the apparent efficacy of brainstem pharmacological treatment which deplete serotonin to produce hyperphagia [3,12]. The present study also showed that raphé lesions did not attenuate the activity reductions produced by fenfluramine. Thus, on two behavioral measures of fenfluramine action, raphé and control animals exhibited similar responsivity. Thus, the present experiment and that of [13], raise serious doubts concerning an indirect seratonergic action of fenfluramine. However, neither the present study nor that of [13] preclude the possibility of a direct serotonergic receptor action of fenfluramine.

The enhanced activity response of rats with raphé lesions to amphetamine agrees with a previous report [11]. Although it has been suggested recently that this facilitation by raphé lesions may be attributed to an elevated activity baseline [8] the close comparability of baseline activity of control and raphé groups in the present experiments tends to indicate that the raphé lesion facilation of amphetamine stimulation cannot always be related to an elevation of baseline activity. The further finding that the raphé lesion enhanced the activity but not the anorexic effect of amphetamine indicates the need to limit statements of drug facilitation or attenuation to the specific response system affected.

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