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Effects of Initial Body Weight on Anorexia and Tolerance to Fenfluramine in Rats

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CARLTON, J. AND N. ROWLAND. *Effects of initial body weight on anorexia and tolerance to fenfluramine in rats.* PHARMACOL BIOCHEM BEHAV **23**(4) 551–554, 1985.—The initial anorexia and development of tolerance to dl-fenfluramine was examined in rats with graded pretreatment body weight losses. Fenfluramine strongly attenuated food intake on the first test day in all weight groups and the magnitude of the initial anorexia did not differ between groups. The time course of anorectic tolerance was also similar in each of the groups. The results do not support a strict "set point" hypothesis according to which the tolerance to fenfluramine anorexia is an artifact of decreasing body weight and/or concurrently increasing relative food deprivation.

dl-Fenfluramine Anorexia Tolerance Body weight "Set point"

PREVIOUS studies have shown that chronic administration of fenfluramine results in the development of tolerance to its anorectic effect [12]. The anorectic action of fenfluramine may be distinguished from other behavioral effects to which tolerance does not develop, such as its discriminative stimulus properties [16]. This dichotomy suggests that anorectic tolerance is "funtional" rather than "dispositional" [14]. The functional interpretation is further supported by direct measurements of brain concentrations of fenfluramine and its metabolite norfenfluramine which are the same after acute or chronic peripheral administration to rats [9].

There are several potential concerns in the interpretation of anorectic tolerance studies in experimental animals [5]. Of relevance to the present study are: (1) tolerance may be contingent on the learned temporal relationship between drug administration and food presentation [4] and (2) changes in the motivation to eat may be brought about by changes in nutritional status resulting from decreased food intake and/or weight loss. The first concern may be discounted because we have found that rats develop full tolerance to fenfluramine anorexia when they receive repeated injections after their daily meal, showing that this tolerance is not contingent on order of meal-drug presentation [13].

Evidence against the second (nutritional) concern is equivocal. Rats show tolerance to fenfluramine anorexia in paradigms which involve neither deprivation nor weight loss such as tail pinch-induced eating [1] and dessert tests [3,12]. However, evidence from a study using rats in a restricted feeding paradigm and treated with a high dose of fenfluramine (20 mg/kg IG) suggested that the tolerance may be an artifact of weight loss [8]. This has been stated more formally by Stunkard [15] as a lowering of body weight "set point" by fenfluramine.

Powley and Keesey [10] provided a paradigm with which to study changes in ingestive behavior relative to an apparent body weight set point. Rats with circumscribed lateral hypothalamic lesions are totally aphagic for several days, lose weight, then recover feeding and maintain a lowered body weight. However, if the rats are food deprived and lose body weight prior to the lesion, they do not show the initial period of total aphagia and lose no further weight.

In the present experiment, we have used a similar paradigm to test the hypothesis that fenfluramine lowers body weight set "set point." We have examined the anorexia to fenfluramine in rats with graded weight loss prior to initial fenfluramine treatment and describe the development of tolerance and weight gain during subsequent free feeding with continued drug administration.

METHOD

Subjects

Female Sprague-Dawley rats (N = 48) initially weighing 185 to 300 g (mean ± SD = 246 ± 21) were housed individually and maintained in a climate controlled vivarium (23°C; 12/12 light/dark cycle). The rats were weighed every 2 days throughout the experiment. Water was freely available at all times.

Pretreatment Feeding Schedules

Rats were first adapted to a diet of powdered chow (Purina

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TABLE 1

BODY WEIGHT AND FOOD INTAKE OF SALINE OR FENFLURAMINE (FEN)-TREATED RATS ON THE FIRST DAY OF INJECTION

Food Schedule Group	Body Weight			Food Intake (g)		
	Preschedule Weight	% Starting Weight	% Ad Lib Weight	Saline	FEN	Anorectic Efficacy*
24 hr	246 ± 8†	110 ± 2†	(100)	25.6 ± 1.6†	7.0 ± 2.2	27%
8 hr	247 ± 9	96 ± 1	(87)	22.8 ± 2.7	5.6 ± 2.7	25%
4 hr	247 ± 5	87 ± 1	(79)	16.4 ± 1.9	5.6 ± 1.3	34%
2 hr	246 ± 6	82 ± 1	(75)	15.3 ± 0.5	7.3 ± 1.7	48%

* Intake of FEN group/intake of saline group.
† Mean ± SE.

No. 5001) offered ad lib in glass jars. They were then divided into four groups matched for body weight and placed on one of four feeding schedules: (1) 2 hr/day (n = 18); (2) 4 hr/day (n = 10); (3) 8 hr/day (n = 10); (4) ad lib (n = 10). During this phase of the experiment, food access was such that the feeding period for each group (except the 24 hr group) ended at 6 p.m. At this time, food cups were removed and weighed and food was returned to the 24 hr group. Saline injections (0.9% NaCl, 1 ml/kg, IP) were also given at this time. After 7 days on these schedules, baseline food intake was determined for each animal over a period of 5 days to verify that the day-to-day intakes were stable. These schedules were designed to produce graded levels of body weight maintenance relative to ad libitum controls.

Fenfluramine Treatment

Fenfluramine treatment was begun after rats had been on the feeding schedules for 12 days. Schedule groups were further divided into drug treatment and vehicle control groups matched for baseline intake. In the 2 hr/day food access group, 6 rats received intraperitoneal injections of 5 mg/kg dl-fenfluramine hydrochloride (A. H. Robins Co.) and 12 rats received saline injections. Six of the rats in this saline-treated group were subsequently allowed to feed ad lib while the other 6 were pair-fed to the fenfluramine-treated rats. In each of the other schedule groups, 5 rats received daily fenfluramine and 5 received saline. All rats in these groups were later allowed to feed ad lib.

On the third day after the initial treatment all rats except the pair-fed group were returned to ad libitum food. Food cups were weighed and refilled daily between 5:30 and 6:30 p.m., approximately 2 hours before lights were turned off in the colony. Rats in the pair-fed group received a measured amount of food equal to the amount eaten on the previous day by the fenfluramine-treated rats to which they were paired. Rats were weighed at this time every other day. Fenfluramine or saline injections were given daily when these maintenance procedures were completed. Treatment was continued for 18 days.

Statistics

Food intake and body weights were compared among groups by ANOVA and TUKEY post-hoc tests. Comparisons were made at various times over the course of the experiment.

RESULTS

The different feeding schedules had the intended effects upon maintenance body weights. The lowest level was associated with the 2 hr schedule, $F(3,45) = 121.8$, $p<0.001$ and all between group comparisons were significant (see Table 1 and Fig. 1). The food intakes following saline on the first test day (Table 1 and Fig. 2) were no different from those during the baseline period, and were related to the duration of food access. Fenfluramine strongly attenuated food intake on the first test day in all groups (Table 1 and Fig. 2). Neither the intake as percentage of the corresponding saline group, $F(3,16) = 1.31$, $p<0.61$, nor the absolute intake in grams, $F(3,16) = .91$, $p<0.45$, were significantly different between schedule groups (Table 1). Therefore, the null hypothesis that the effect of fenfluramine is the same in all groups cannot be rejected.

Fenfluramine continued to suppress intake significantly in all groups on days 2 and 3. During this ad lib refeeding, all previously restricted groups gained weight (Fig. 1). The data were analyzed for drug versus saline differences within a group. Rats which had been on the 2 hr feeding schedule had significant differences in body weight on the first 5 days of ad lib food (day 1: $F(2,17) = 9.56$, $p<0.002$; day 3: $F = 5.29$, $p<0.02$; day 5: $F = 4.51$, $p<0.03$). Both the fenfluramine-treated group and the pair-fed group had body weights that were significantly lower than the saline group over this period ($p<0.05$). Fenfluramine-treated rats which had previously been on the 24 hr feeding schedule also had significantly lowered body weights over this period. By day 7, body weights of rats in the different treatment groups were not significantly different. Weights of saline- versus drug-treated rats in the 4 and 8 hr schedule groups were not significantly different at any time.

DISCUSSION

Fenfluramine produced a 50–75% decrease in food intake of drug naive animals. Drug efficacy was not significantly affected by body weight. This was not expected according to a "set point" hypothesis. Graded levels of prior body weight reduction would be expected to attenuate or abolish the initial anorexia to fenfluramine, and the animals should appear "tolerant" to the drug. The time course of anorectic tolerance was also similar in each of the 4 groups despite differences in initial body weight.

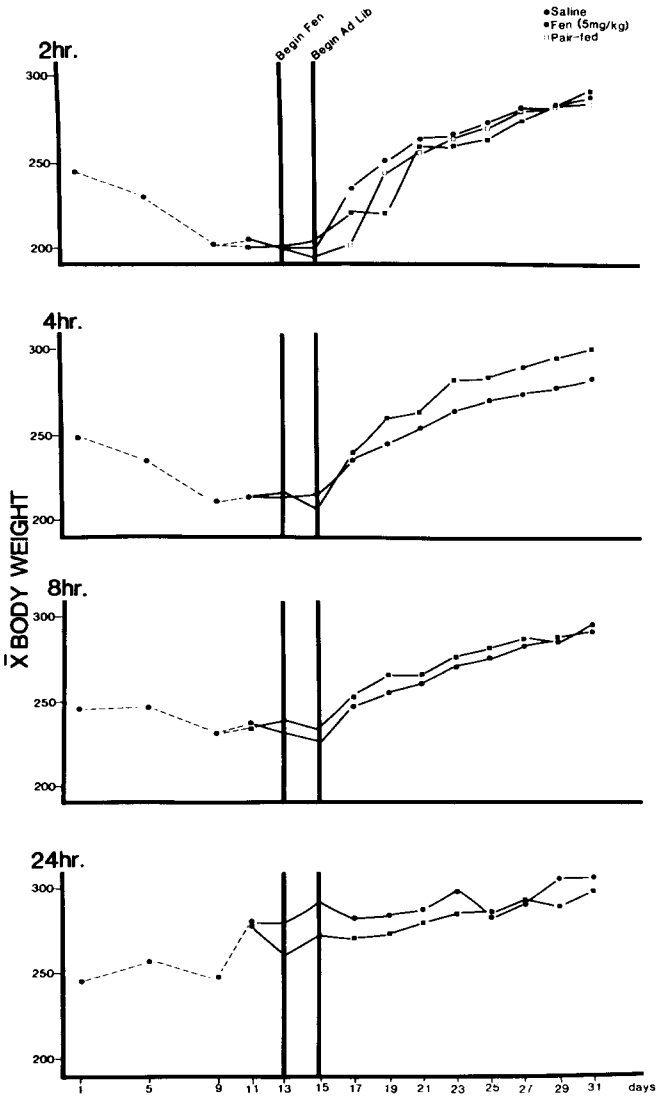

FIG. 1. Group mean body weights of the various groups during the experiments. The left part of each panel shows the weight changes during the indicated period of scheduled food access per 24 hr. The groups were divided; half received fenfluramine (FEN, 5 mg/kg) at the first vertical line (day 13) and remained on the schedule until day 15, then all rats were fed ad libitum except the pair fed group in the top panel (open squares). The injections were made daily throughout this period of refeeding.

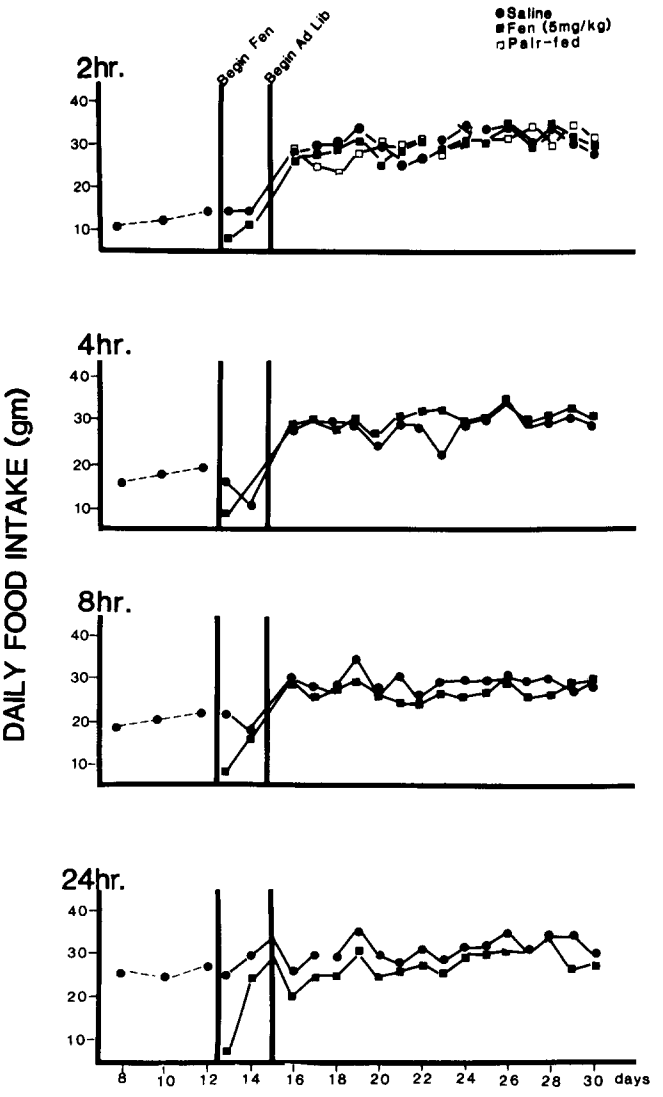

FIG. 2. Mean food intakes of the various groups during the experiment. See legend to Fig. 1 for details.

These results suggest that tolerance to fenfluramine anorexia is not due to decreased body weight or concurrently increasing food deprivation. In a previous study, Levitsky *et al.* (1981) arrived at different conclusions [8]. Their procedures were considerably different from ours and the dosage of fenfluramine was 20 mg/kg IG (compared to 5 mg/kg IP in the present experiment). While we cannot be sure of the critical differences between the two experiments, it should be noted that complete anorectic tolerance does not develop to high doses of fenfluramine [1] and it has been suggested that high doses may be neurotoxic [6]. The 5 mg/kg fenfluramine used in the present study and the 50–75% anorexia appear to be more relevant to the clinical situation in which 1–2 mg/kg/day is a typical dosage. Our results certainly suggest that there are limits to the generality of the set point hypothesis of weight regulation following fenfluramine, at least as studied in this paradigm. The lowered set point in lateral hypothalamic lesion rats [10] has also been questioned in its interpretation [11].

However, other data from our laboratory suggest that the lowered set point notion may be a better description of fenfluramine's effects in other paradigms. We have compiled data from ad lib (chow) feeding rats of varying strain, sex and age obtained over a period of several years. It is apparent that the ability of a daily dose of 5 mg/kg fenfluramine to produce net weight loss is dependent upon the initial body

weight of the rat (r = .96). Thus rats with initial weights above 300 g typically lose weight while those with initial weights below 300 g will gain weight. Vehicle injected controls did not lose weight. These data suggest that some modified form of the set point hypothesis may be true in heavier rats. This may also be the case in obese humans which do not develop apparent tolerance to fenfluramine-induced weight loss as quickly as do lean experimental animals (c.f. [7]). Fenfluramine has effects on lipid metabolism in vitro (reviewed in [2]), and these effects on adipose tissue may account for some of its differential effects in obese versus lean subjects.

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