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The Effects of d-Fenfluramine on Saccharin Intake and Preference, and on Food and Water Intake

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FLETCHER, P. J. *The effects of d-fenfluramine on saccharin intake and preference, and on food and water intake.* PHARMACOL BIOCHEM BEHAV **29**(4) 687–691, 1988.—The effects of d-fenfluramine on saccharin intake and preference were examined to investigate whether the reduced rate of eating induced by this compound reflects a reduction in the palatability of foods. In two separate experiments, water deprived rats were offered a choice between a 0.05% solution of saccharin and water, or a 0.2% saccharin solution and water. Injection of d-fenfluramine at doses which reliably decreased food intake resulted in dose dependent reductions in total fluid intake and saccharin intake. A trend towards reduced water intake was observed also, and this together with the reduced saccharin intake resulted in no overall change in saccharin preference. In a further experiment, d-fenfluramine reduced the water intake of water deprived rats to the same extent as it reduced total fluid intake in the choice tests. Since d-fenfluramine failed to alter saccharin preference, it is unlikely that the slowed eating rate induced by this compound indicates a reduction in food palatability. Instead, it is likely that this behaviour results from a subtle motor deficit, such that fenfluramine treated animals are unable to maintain ingestion at the same rate as non-drugged animals. This explanation could account for the reduction in the consumption of non-nutritive saccharin solutions and water in water deprived animals. The relevance of this action to the anorectic effect of fenfluramine is discussed.

d-Fenfluramine Food intake Water intake Saccharin preference Eating rate Rats

THE pharmacological and behavioural mechanisms of action of the anorectic drug fenfluramine have been the subject of many experiments ([11, 16, 17] for reviews). It is generally assumed that the anorectic action of fenfluramine is due to the ability of this drug to release and inhibit the re-uptake of 5-hydroxytryptamine (5-HT; serotonin) [11,12] leading to an increase in serotonergic neurotransmission. At the behavioural level it has been reported consistently that fenfluramine reduces meal and bout size in free feeding [2, 5, 8, 9, 14] and food deprived rats [3,10]. In free feeding rats this effect is associated with a prolonged inter-meal interval [5,9] and the occurrence of the behavioural sequence of satiety [5]. These results have led to the proposal that fenfluramine hastens the onset of satiety [1, 4, 5, 9] and that 5-HT is involved in the expression of normal satiety [1,9]. However, in addition to reducing meal and bout size, fenfluramine also induces a marked reduction in eating rate [2, 3, 8, 10]. This effect of fenfluramine is difficult to reconcile with the satiety hypothesis since it occurs across the entire meal [8], and since a slowed eating rate is not a characteristic of the development of normal satiety [7,8].

Eating rate is reduced also by the addition of quinine to the diet [6], and by treatment with the neuroleptic pimozide [2,3]. Quinine adulteration obviously renders food less palatable, whilst it has been suggested that pimozide may blunt the hedonic value of positive reinforcers such as food [21]. Although motor impairments have been implicated in the behavioural effects of pimozide (e.g., [13]), experiments using food-related stimuli have revealed an apparently specific anhedonic effect. Thus, pimozide was found to reduce the intake of a palatable saccharin-glucose solution to a greater extent than it reduced water intake [25]. In a two-bottle choice test (sucrose versus water) pimozide decreased sucrose preference by reducing sucrose intake and increasing water intake [19,20]. Therefore, the reduced eating rate induced by pimozide may reflect a reduction in the palatability of food.

The present experiment was designed to investigate whether the slowed eating rate induced by fenfluramine indicates a reduction in the palatability of food, by examining the effects of fenfluramine on the preference for highly preferred, and marginally preferred saccharin solutions.

The majority of previous experiments have used the racemic form of fenfluramine. In the present experiment, d-fenfluramine, which is approximately twice as potent as d,l-fenfluramine, was used. However, there is no evidence that the behavioural effects of the two compounds are qualitatively different [17].

METHOD

Subjects

Forty-eight male Sprague-Dawley rats weighing 230–250 g at the start of the experiments served as subjects. The

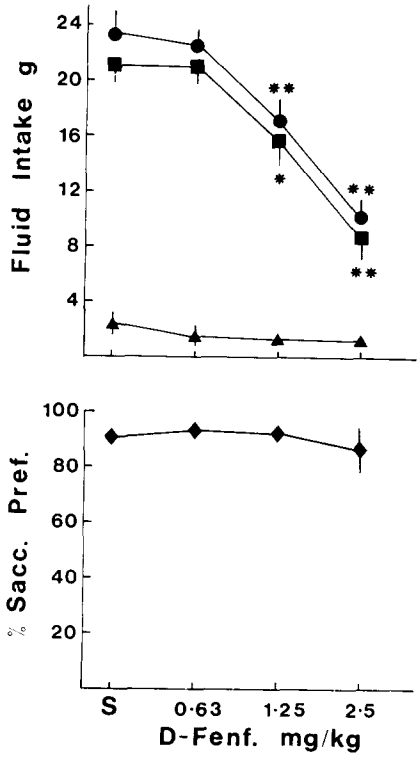

FIG. 1. The upper panel shows the effects of d-fenfluramine on 0.2% saccharin intake (squares), water intake (triangles), and total fluid intake (circles). The lower panel shows saccharin intake as a percentage of the total fluid intake. Each point represents the mean (±S.E.M.) intake of 10 rats. *Differs significantly from saline, $p<0.05$. **Differs significantly from saline, $p<0.01$.

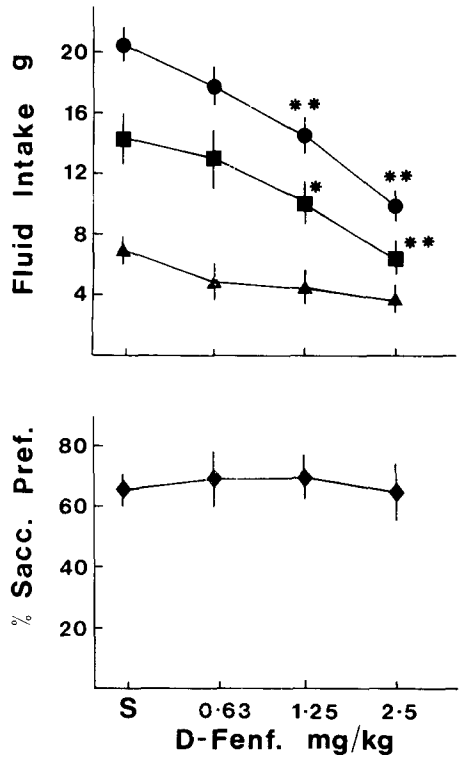

FIG. 2. The upper panel shows the effects of d-fenfluramine on 0.05% saccharin intake (squares), water intake (triangles) and total fluid intake (circles). The lower panel shows saccharin intake as a percentage of the total fluid intake. Each point represents the mean (±S.E.M.) intake of 8 rats. *Differs significantly from saline, $p<0.05$. **Differs significantly from saline, $p<0.01$.

animals were housed individually in hanging wire mesh cages in a temperature controlled room (20±2°C) under a 12-hour light/dark cycle (lights on at 8 a.m.). Food and water were available at all times prior to the beginning of the test.

Saccharin Preference Testing

Forty rats were placed on a water deprivation schedule, with water available for 1-hour daily beginning at 2:00 p.m. Food was withdrawn during this period. Following 3 days of restricted water access, each animal was presented with 2 bottles during the drinking session, one containing water and the other containing a solution of 0.2% (w/v) sodium saccharin. This procedure was continued for 9 days with the bottle positions being reversed daily to minimize the development of position habits. Each day fluid intakes were determined by weighing the bottles before and after the sessions. Saccharin preference scores were determined by dividing the saccharin intake (g) by the total fluid intake (g) and multiplying the result by 100%. On the following day the animals were assigned randomly to 4 groups (n=10 each) and then injected (IP) with 1 ml/kg 0.9% saline, 0.63, 1.25 or 2.5 mg/kg d-fenfluramine hydrochloride (Servier). Thirty minutes later one bottle containing water and one bottle containing 0.2% saccharin were presented for 1 hour; the bottle positions were reversed 5 minutes after the initial presentation. Fluid intakes and saccharin preference scores were determined at the end of the test.

For the following week, the rats were maintained on the same deprivation schedule, but with only water available for the 1-hour access period. The animals were then presented daily with two bottles, one containing water and one containing 0.05% (w/v) saccharin for 7 days. Eight rats were eliminated from the study at this point for failing to show stable day to day saccharin intake and preferences. On the following day the remaining rats were assigned randomly to 4 groups (n=8 each) which were adjusted for baseline saccharin preferences. Drug administration and fluid intake measurement procedures were exactly as described above.

Water and Food Intake Studies

Eight rats were selected at random from the pool of animals used in the preference experiments. These animals were allowed free access to water for 7 days following the completion of the saccharin preference studies. They were then re-adapted to the 1-hour daily water access schedule. Once water intakes had stabilized the animals were injected (IP) with 1 ml/kg 0.9% saline, 0.63, 1.25 or 2.5 mg/kg d-fenfluramine. Water was then presented thirty minutes later, and consumption determined over 1 hour. Each rat received every dose of fenfluramine and saline in a counterbalanced order with 3 drug free days between successive treatments.

Eight experimentally naive rats were used to examine the effects of d-fenfluramine on food intake. These animals were trained to consume their daily food intake ration in a 4-hour period beginning at 1:00 p.m. When daily food intakes had stabilized the rats were injected (IP) with 1 ml/kg 0.9% saline, 0.63, 1.25 or 2.5 mg/kg d-fenfluramine, thirty minutes prior to being presented with a weighed amount of food.

TABLE 1

EFFECTS OF d-FENFLURAMINE ON FOOD INTAKE AND FLUID INTAKE

		d-Fenfluramine mg/kg		
	Saline	0.63	1.25	2.5
Food intake g	9.3 (±0.4)	6.6 (±0.6)* 71%[a]	5.9 (±0.7)† 63.6%	2.4 (±0.6)† 25.4%
Water intake g	19.4 (±0.7)	20.3 (±1.1) 104%	15.1 (±1.1)* 77.9%	8.8 (±1.5)† 45.3%
Total fluid intake g (0.05% sacc. + water)	20.2 (±0.9)	17.7 (±1.2) 87.6%	14.4 (±1.4)† 71.3%	9.9 (±0.9)† 49.0%
Total fluid intake g (0.2% sacc. + water)	23.3 (±0.9)	22.5 (±0.8) 96.6%	17.1 (±1.7)† 73.3%	8.8 (±1.6)† 39.0%

*Differs from saline, $p<0.05$.
†Differs from saline, $p<0.01$.
[a]Intakes expressed as a percentage of saline condition.

Food intakes were determined 1-hour later by weighing the remaining food, plus spillage collected on paper towels placed beneath the cages. Each rat received every dose of fenfluramine in a counterbalanced order with 3 drug free days between successive treatments.

Statistical Analysis

In each experiment the effects of d-fenfluramine on the dependent variables (total fluid intake, water intake, saccharin intake, saccharin preference, and food intake) were analyzed by one-way analysis of variance, followed by Dunnett's test for comparisons against a control mean.

RESULTS

Saccharin Preference

The effects of d-fenfluramine on the intake of, and preference for, 0.2% saccharin are shown in Fig. 1. d-Fenfluramine induced clear dose dependent decreases in total fluid intake, $F(3,36)=20.3$, $p<0.001$, and saccharin intake, $F(3,36)=15.4$, $p<0.001$. The apparent reduction in water intake was not significant, $F(3,36)=0.3$, $p<0.1$, presumably because the low level of water intake under saline treatment represents a near floor against which it is difficult to detect significant reductions. However, the non-significant decreases in water intake induced by 1.25 and 2.5 mg/kg d-fenfluramine (to approximately 60% of the intake under saline treatment) were sufficient to offset the marked reduction in saccharin intake so that saccharin preference scores were not changed by d-fenfluramine, $F(3,36)=0.1$, $p<0.1$.

Figure 2 depicts the effects of d-fenfluramine on the intake of, and preference for, 0.05% saccharin. d-Fenfluramine induced dose dependent reductions in total fluid intake, $F(3,28)=14.1$, $p<0.001$, and saccharin intake, $F(3,28)=4.4$, $p<0.025$. The effect of d-fenfluramine on water intake just failed to reach the conventional level of significance of $p=0.05$, $F(3,28)=2.88$, $p<0.052$. However, a comparison of the data from the saline and 2.5 mg/kg d-fenfluramine groups revealed this difference to be significant, $t(14)=2.58$, $p<0.05$. Saccharin preference was not altered by d-fenfluramine, $F(3,28)=0.1$, $p<0.1$.

Food and Water Intake

The effects of d-fenfluramine on food intake in food deprived rats, and water intake in water deprived rats, are shown in Table 1. Both food intake, $F(3,21)=23.8$, $p<0.001$, and water intake, $F(3,21)=21.6$, $p<0.001$, were significantly decreased by d-fenfluramine in a dose dependent fashion. It can be seen also from Table 1 that the d-fenfluramine induced decreases in water intake were of similar magnitude to the reductions in total fluid intake observed in the two-bottle choice tests.

DISCUSSION

The effects of d-fenfluramine on saccharin intake and preference were examined in order to investigate whether the reduced eating rate induced by fenfluramine reflects a reduction in the palatability of food. In two separate choice tests (saccharin versus water) d-fenfluramine markedly reduced total fluid intake and the intakes of 0.05% and 0.2% saccharin in a dose-dependent manner. d-Fenfluramine at 1.25 and 2.5 mg/kg was effective in this respect; 0.63 mg/kg was not effective. Water intake in the choice tests was less noticeably affected by d-fenfluramine. However, these results should not be interpreted as evidence that d-fenfluramine induces a selective reduction in saccharin intake. Firstly, these were apparent trends towards reduced water intake with increasing doses of d-fenfluramine. In the case of the choice between 0.05% saccharin and water this reduction in water intake narrowly missed the conventional level of significance. Water intake of the control group in the choice experiment involving 0.2% saccharin was extremely low, and probably represents a floor effect. Secondly, the reductions in water intake were sufficient to offset the reductions in the intakes of both concentrations of saccharin so that saccharin preference was not affected by d-fenfluramine. Thirdly, the administration of d-fenfluramine to 23-hour water deprived rats reduced water intake to the same extent as it reduced fluid intake in the choice experiments. It is particularly important to note that rats treated with 1.25 and 2.5 mg/kg d-fenfluramine did not increase their water intake to compensate for the reduced saccharin intake.

The results of the choice experiments clearly demonstrate that d-fenfluramine does not reduce the preference of a marginally preferred (0.05%) or a highly preferred (0.2%) saccharin solution. Thus, it is unlikely that the anorectic action of fenfluramine, and the decreased eating rate, derives from a reduction in the palatability of foods. It has been shown

previously that fenfluramine induces a mild reduction in spontaneous motor activity (e.g., [14]), and has been suggested that the fenfluramine induced slowing of eating rate is due to an inability of the drugged animal to generate high response rates [8]. In support of this hypothesis is the observation that fenfluramine in the dose range 0.3–3 mg/kg decreased operant response rate on schedules of reinforcement which maintained high levels of control responding [15]. This type of motor deficit could account for the decreased saccharin and water intakes observed in the present experiments, since a long period of water deprivation induces a high rate of intake when fluids are subsequently made available. Similarly, the impaired performance of d-fenfluramine treated rats in a food reinforced runway paradigm [18] may result from this motor deficit.

It has been reported that fenfluramine selectively suppresses carbohydrate intake in rats [22] and that it suppresses carbohydrate intake and cravings in obese humans [23,24]. The present results showing that d-fenfluramine does not alter the preference for saccharin suggest that these effects of fenfluramine are not related to a reduction in the preference for sweet carbohydrates. This conclusion is supported also by the finding that rats treated with fenfluramine reduce their carbohydrate intake regardless of the relative sweetness of the carbohydrate source [22].

Several studies have shown that fenfluramine does not impede the initiation of feeding [3,4], reduces meal and bout size [2, 3, 5, 8–10, 14], prolongs the inter-meal interval [5,9], and induces behaviour similar to that induced by satiety [5]. This evidence has been interpreted as evidence for a highly specific action of fenfluramine on the processes of satiation and satiety. The present results, however, show that the effects of fenfluramine on ingestion are not restricted to food intake, since the intakes of water and solutions of non-nutritive saccharin were reduced, albeit to a lower degree. Thus, it appears that fenfluramine exerts multiple effects on ingestive behaviour. Some of these effects may reflect a direct action on feeding motivation, but a non-specific action which may be expressed as a reduced eating rate also contributes to the anorexia induced by fenfluramine.

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