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Comparisons Between the Effects of 5-HT and DL-Fenfluramine on Food Intake and Gastric Emptying in the Rat

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FRANCIS, J., D. CRITCHLEY, C. T. DOURISH AND S. J. COOPER. *Comparisons between the effects of 5-HT and DL-fenfluramine on food intake and gastric emptying in the rat.* PHARMACOL BIOCHEM BEHAV **50**(4) 581–585, 1995. – 5-Hydroxytryptamine (5-HT) dose-dependently increased gastric emptying in rats, whereas DL-fenfluramine produced a biphasic dose response curve for gastric emptying. Thus, fenfluramine increased gastric emptying at a dose of 0.1 mg/kg but decreased it at doses of 1 and 3 mg/kg. Both 5-HT and DL-fenfluramine produced significant decreases in food intake. As 5-HT produced opposing effects on gastric emptying and food intake, it appears that the anorectic effect of 5-HT may not depend on changes in the rate of gastric emptying. In contrast, doses of DL-fenfluramine that decreased feeding also decreased gastric emptying, which suggests that reduced food intake may at least partly result from decreased gastric emptying. Taken together, these results suggest that the effects of 5-HT and DL-fenfluramine on feeding and gastric emptying may be mediated by different mechanisms.

5-HT DL-Fenfluramine Food intake Gastric emptying Rat

THERE have been several proposals that the effects of serotonergic drugs on food intake depend on their effects of gastric emptying. These suggestions imply that 5-hydroxytryptamine (5-HT)-dependent anorexia follows from a peripheral effect to slow down the rate of gastric emptying. It is well established that peripheral administration of 5-HT itself reduces food intake in rats under a variety of experimental conditions (3,12,13,15). Because 5-HT does not cross the blood-brain barrier (14), this effect is presumably mediated by peripheral 5-HT receptors. Further support for a peripheral site of action for 5-HT induced anorexia comes from studies showing that this effect is attenuated by the peripheral 5-HT receptor antagonist, xylamadine (3,6). Moreover, Fletcher and Burton demonstrated that peripheral 5-HT (2 mg/kg, SC) reduced the rate of gastric clearance of a wet mash meal (equal weights of chow and water) in rats (7).

It has also been proposed that the anorectic effect of the indirect 5-HT agonist DL-fenfluramine follows from a peripheral action to reduce the rate of gastric emptying (1,2,4,19). Davies and colleagues demonstrated that DL-fenfluramine (5 mg/kg, IP) inhibited gastric clearance of a 5-g meal of wet mash (equal weights of chow and water) in 18-h food-deprived rats (4). The size of the DL-fenfluramine effect on food intake was related to the amount of food emptied from the stomach before the drug treatment. They suggested that this indicated that the anorectic effect of DL-fenfluramine is due, at least in part, to the inhibition of gastric emptying. Rowland and Carlton replicated these results in 20- and 22-h food-deprived rats (19). In addition, tolerance has been shown to develop to DL-fenfluramine-induced anorexia (18) and to its inhibition of gastric emptying (19). Rowland and Carlton (20) noted that there was a close temporal and quantitative relationship between the development of tolerance to these measures. However, this correlation does not demonstrate a causal link be-

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tween DL-fenfluramine's effect on gastric emptying and its effect on satiety.

To determine whether the lengthy periods of food deprivation employed in the previous experiment affected DL-fenfluramine's inhibition of gastric emptying, Booth and colleagues investigated the effects of DL-fenfluramine (5 mg/kg IP) on gastric clearance of chow in 4-h food-deprived and in freely fed rats (2). In both conditions, DL-fenfluramine significantly reduced the rate of gastric emptying. Hence, the ability of DL-fenfluramine to inhibit gastric emptying is independent of the degree of food deprivation. Baker and colleagues reported that the inhibition of gastric emptying produced by DL-fenfluramine (2.5 mg/kg, IP) was attenuated by xylamadine (1). This result indicated a peripheral site of action for DL-fenfluramine's effect.

DL-Fenfluramine has been shown to affect gastric emptying in species other than the rat. Robinson and co-workers observed that DL-fenfluramine (2 mg/kg) administered intragastrically reduced the rate of gastric clearance of sucrose solution meal in 17-h food-deprived Rhesus monkeys (*Macaca mulatta*) (17). The degree of inhibition of glucose intake was found to be strongly related to the degree of inhibition of gastric emptying. This result again suggests an important involvement of gastric emptying in DL-fenfluramine-induced anorexia. Horowitz and colleagues reported that 40 mg/kg DL-fenfluramine administered orally reduced the rate of gastric emptying in both normal-weight and obese patients (10). In these experiments comparisons were drawn between the effects of 5-HT and DL-fenfluramine in tests of anorexia and gastric emptying. Based on earlier studies, the prediction is that 5-HT and DL-fenfluramine should produce similar effects on each of these two measures. However, it should be noted that the effects of the two drugs have not previously been compared under similar experimental conditions. In addition, previous work on the effects of 5-HT and DL-fenfluramine have investigated the effects of only a narrow dose range for each compound. Hence, the present experiments incorporated a much wider dose range for each drug.

EXPERIMENT 1: EFFECTS OF 5-HT AND DL-FENFLURAMINE ON THE RATE OF GASTRIC EMPTYING IN 17-H FOOD-DEPRIVED RATS

Materials and Methods

Animals. Seventy-four adult, male Sprague-Dawley rats (Charles River, Manston, UK), weighing 250–300 g at the time of testing, were housed individually with ad lib access to food pellets (Diet 41B; Heygate and Sons, Northants, UK). They were maintained under a 12 L : 12 D cycle with lights on at 0800 h. Prior to testing, animals were transferred to test cages, where they were food deprived overnight (ca. 17 h) with ad lib access to water.

Drugs. DL-Fenfluramine hydrochloride and 5-HT creatinine sulphate were purchased from Sigma Chemical Co. Ltd. (Poole, UK). Both drugs were dissolved in isotonic saline and injected intraperitoneally. Drugs were administered in a volume of 1 ml/kg, 20 min before the start of the feeding tests (doses refer to the base).

Test meal. Carboxymethylcellulose (low viscosity, 10–20 cps, Sigma Chemical Co. Ltd.) was added in four 5-g portions (20 g in total) to 250 ml of distilled water. After the addition of each portion, the mixture was agitated in a blender for 1 min. Casein (bovine milk; Sigma Chemical Co. Ltd.) was added to this mixture in two 8-g portions (16 g in total), followed by 8 g of cane sugar (household; Tate and Lyle, London, UK) and 8 g of cornstarch (Sigma Chemical Co. Ltd.). Following the addition of each portion of these ingredients, the mixture was agitated for 1 min. The resulting mixture was a white semisolid paste, which was placed in a refrigerator overnight to allow any air trapped in the mixture to escape. Two hours before use, the mixture was removed from the refrigerator and allowed to reach room temperature.

Procedure. The rats were injected 20 min before receiving the test meal. They were then given by gavage a weighed amount of test meal (ca. 3 g) equalling 3 ml in volume. Thirty minutes later, the rats were killed by cervical dislocation and

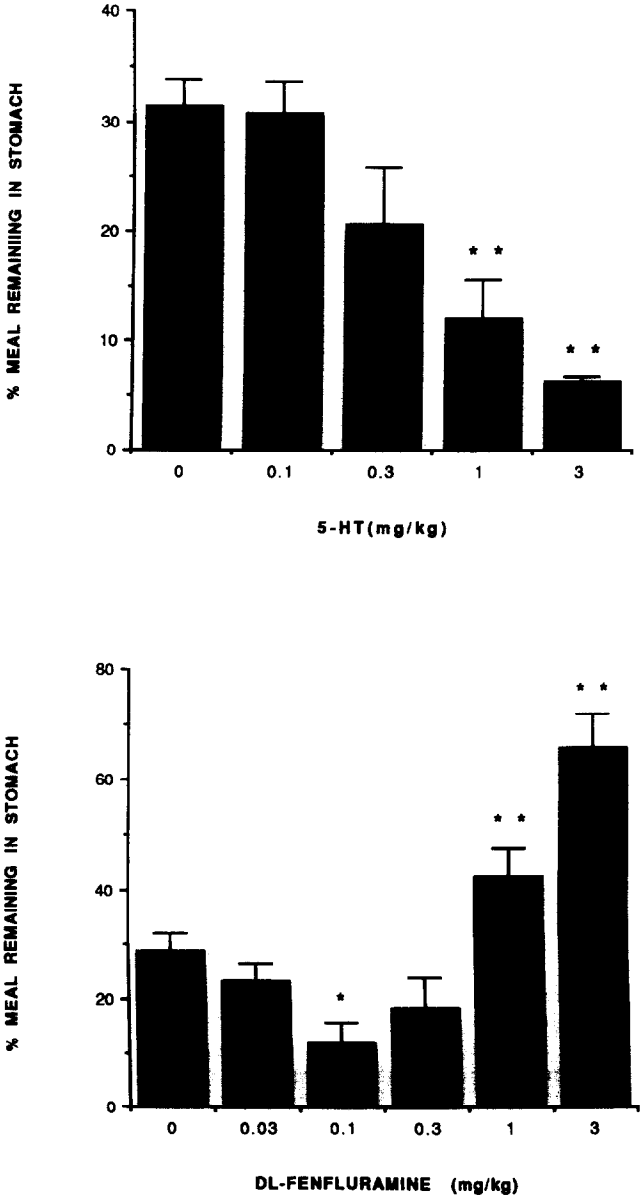

FIG. 1. Effects of 5-HT (0.1–3 mg/kg) (upper panel) and DL-fenfluramine (0.03–3 mg/kg) (lower panel) on gastric emptying in 17-h food-deprived rats ($N \leq 6$ per condition). Results are shown as percentage of test meal remaining in the stomach ± SEM after 30 min. Levels of significance for comparisons with vehicle condition: $^{*}p < 0.05$; $^{**}p < 0.01$ (Dunnett's *t*-test).

laparotomized. The stomachs were ligated at the oesophagus and pylorus to prevent leakage, removed, and weighed. The stomachs were then opened, and any remaining test meal was rinsed out. Any excess water was removed from the stomach by blotting on the tissue paper. The empty stomachs were then weighed. The amount of the test meal that had remained in the stomach was then calculated by subtraction of successive weighings. The percentage of 3 g test meal remaining in the stomach after 30 min was calculated. Copraphagic animals or rats with food particles remaining in their stomachs were excluded from the results. Data were analysed using one-way ANOVA for independent samples followed by Dunnett's *t*-test.

Results

As Fig. 1 (upper panel) shows, 5-HT (0.1–3 mg/kg) dose-dependently increased the rate of gastric emptying in 17-h food-deprived rats [$F(4, 28) = 8.1, p < 0.002$]. Dunnett's *t*-test revealed that doses of 1 and 3 mg/kg significantly increased the rate of gastric emptying. At 3 mg/kg, 5-HT reduced the amount of test meal remaining in the stomach to 33.6% of the control value.

DL-Fenfluramine produced a biphasic dose response curve for gastric emptying. As Fig. 1 (lower panel) shows, there were significant reductions in the rate of gastric emptying at 1 and 3 mg/kg. These doses increased the amount of the test meal remaining in the stomach by 48.2 and 129.6% of control values, respectively. At 0.1 mg/kg DL-fenfluramine significantly increased the rate of gastric emptying, with a reduction in test meal remaining in the stomach to 41.4% of control value.

EXPERIMENT 2: EFFECTS OF 5-HT AND DL-FENFLURAMINE ON THE CONSUMPTION OF STANDARD DIET IN 17-H FOOD-DEPRIVED RATS

Materials and Methods

Animals. Ninety-eight adult, male Sprague–Dawley rats, weighing 250–300 g, were housed under conditions described previously and tested in their home cages. Prior to testing they were food-deprived overnight (ca. 17 h) with ad lib access to water.

Procedure. Following the administration of drugs, the rats were allowed access to a weighed amount of their standard diet (Heygate 41B; Heygate and Sons, Northants, UK). The diet was briefly removed, reweighed, and replaced 30 and 60 min after presentation. Any spillage was collected and appropriate corrections were made to weighings. Food intake was measured to the nearest 0.1 g and calculated by subtraction of successive weighings. Data were analysed using one-way ANOVA for independent samples followed by Dunnett's *t*-test.

Results

5-hydroxytryptamine significantly reduced food intake in food-deprived rats after 30 min [$F(4, 45) = 4.8, p < 0.0027$]. As Fig. 2 (upper panel) shows, 5-HT dose dependently reduced intake. Vehicle-treated animals consumed an average of 4.3 and 6.2 g after 30 and 60 min, respectively. At 1 and 3 mg/kg, 5-HT decreased food intake by 62.7 and 79.7% of the control values, respectively. After 60 min, 5-HT significantly reduced food intake [$F(4, 45) = 4.8, p < 0.05$]. At 3 mg/kg, 5-HT decreased food intake by 57.6% of control value.

As Fig. 2 (lower panel) indicates, DL-fenfluramine significantly reduced food intake after 30 min [$F(5, 42) = 7.0, p < 0.001$]. In addition, food intake remained suppressed for 60 min [$F(5, 420 = 9.052, p < 0.001$] and 90 min [$F(5, 42) = 10.028, p < 0.0001$]. Dunnett's *t*-test revealed that doses of 1 and 3 mg/kg significantly decreased consumption throughout the 90-min test. However, after 90 min, 0.1 mg/kg DL-fenfluramine did not reduce food intake, but produced a small increase in consumption (Fig. 3).

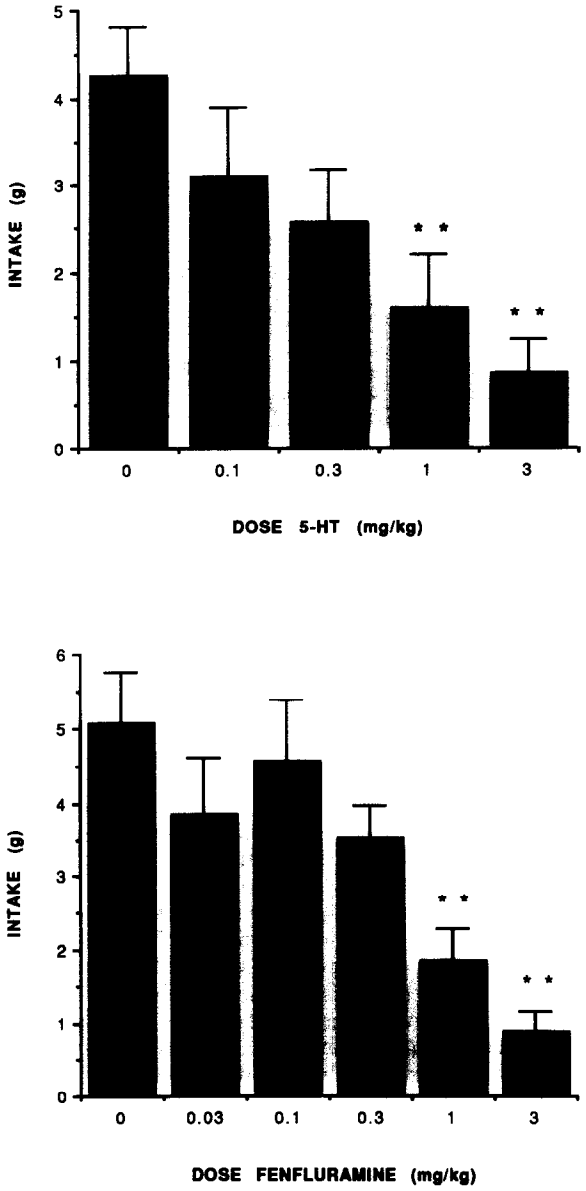

FIG. 2. Upper panel: Effect of 5-HT (0.1–3 mg/kg) on food intake in 17-h food-deprived rats ($N = 10$ per condition). Lower panel: Effect of DL-fenfluramine (0.03–3 mg/kg) on food intake ($N = 8$ per condition). Results are shown as mean intake (g) ± SEM after 30 min. Levels of significance for comparisons with vehicle condition: $^{*}p < 0.05$; $^{**}p < 0.01$ (Dunnett's *t*-test).

DISCUSSION

These results show that 5-HT dose dependently increased gastric emptying, but decreased food intake in 17-h food-

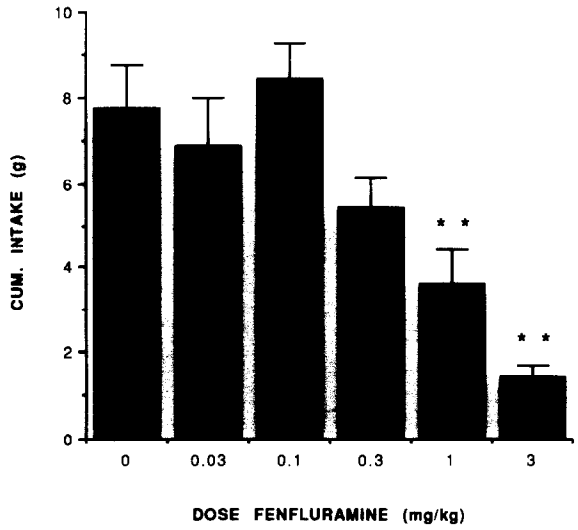

FIG. 3. Effect of DL-fenfluramine (0.03–3 mg/kg) on food intake (N = 8 per condition). Results are shown as mean intake (g) ± SEM after 90 min. Levels of significance for comparisons with vehicle condition: $*p < 0.05$; $**p < 0.01$ (Dunnett's t-test).

deprived rats. The dose-dependent decrease in food intake obtained with 5-HT is consistent with earlier studies (3,7,15). The dose-dependent increase in gastric emptying produced by 5-HT was contrary to what may have been expected. Thus, Fletcher and Burton reported that, under their experimental conditions, 5-HT decreased the rate of gastric clearance (7). Gullikson and colleagues (1991), however, found that 5-HT both increased and decreased gastric emptying in food-deprived rats depending on the dose (8). Their method was also based on that developed by Droppleman and colleagues (5), which was used in this study. Gullikson and co-workers found that both low and high doses of 5-HT (0.3 and 30 mg/kg, IP) decreased gastric emptying, whereas doses in the range of 1–10 mg/kg increased the rate of gastric emptying (8). The results of the present study are in partial agreement with those of Gullikson and co-workers, because an increase in gastric emptying was also observed at 1 and 3 mg/kg of 5-HT. However, at doses < 1 mg/kg, there was no decrease in gastric emptying rate, as they reported. In addition, Prove and Ehrlien observed that 5-hydroxytryptophan (5-HTP), a precursor of 5-HT, increased the rate of gastric emptying in 18-h food-deprived dogs (16). Intravenous infusions of 5-HTP (200 μg/kg per min) increased the rate of emptying of a mashed potato meal that had been given by an oral-gastric tube into the stomach. Thus, there appear to be instances where 5-HT produces an increase in the rate of gastric emptying.

An explanation for the difference in results between the present study and those of others may be that action of 5-HT at individual or a combination of its receptor subtypes produces different effects on gastric emptying through the several processes that contribute to this process. The recruitment of a particular receptor subtype may depend on the type of food, as this may vary in terms of either macronutrient composition or consistency. The net effect of 5-HT in gastric emptying may therefore depend on several factors.

The macronutrient composition of the food may influence gastric emptying. Kanarek and Dushkin (11) showed that peripheral administration of 5-HT (2–6 mg/kg, IP) selectively reduced fat intake in food-deprived and freely feeding rats (11). They noted that, compared to other macronutrients, fat prolongs gastric emptying. Thus, consumption of fat may have an additional effect in association with 5-HT, and it is possible that the slowing down of gastric emptying produced may lead to the rat avoiding fat. The effects of 5-HT on gastric emptying may therefore depend on the type of macronutrient consumed. Because the test meal used the present study did not contain fat, its emptying may therefore be different from one with a fat-containing meal. Fletcher and Burton (7) investigated the emptying of a meal containing carbohydrates, protein, and fat. Thus, the reduction in gastric emptying produced by 5-HT that they observed may have depended on the fat content in the meal (7).

Hinder and Kelly demonstrated that there are different patterns of emptying for liquids and solids. Liquids are emptied almost immediately after ingestion, whereas solids are retained in the stomach until they are broken down into chyme, after which they are emptied at a rate similar to that for liquids (9). This suggests that food entering the small intestine needs to be of a semisolid consistency. Fletcher and Burton examined the effects of 5-HT on the emptying of a solid meal and found that 5-HT reduced the rate of emptying (7). Theoretically, this may be the expected effect of 5-HT, promoting satiety through gastric distention as well as causing the retention of food in the stomach to be broken down into chyme. However, in the present study, the test meal was of a semisolid consistency, not requiring prolonged retention in the stomach. Under these conditions 5-HT may affect gastric emptying in a different way to promote satiety. For example 5-HT may act to increase the rate of emptying, causing the rapid entry of food into the duodenum and producing satiety through duodenal mechanisms.

Fletcher and Burton (7) demonstrated that methysergide reversed 5-HT-induced anorexia but had no effect on 5-HT-induced suppression of gastric emptying. They suggested that 5-HT anorexia is independent of any action on gastric emptying. The results of this present study would also suggest such a dissociation between the effects of 5-HT on these two measures.

DL-Fenfluramine exhibited a biphasic dose-response curve for gastric emptying, but reduced food intake in food-deprived rats. More specifically, at doses that decreased gastric emptying, there was a corresponding reduction in food intake. This gives partial support to the hypothesis of Davies and colleagues (4), that the anorectic effect of DL-fenfluramine may depend on changes in gastric emptying. Rowland and Carlton (20) examined the anorectic action of DL-fenfluramine (5 mg/kg, IP) on food intake in sham-feeding rats. In the sham-feeding preparation, food does not accumulate in the stomach, and therefore it is unlikely that gastric distention plays any significant role in this paradigm. They observed that DL-fenfluramine initially suppresses intake; however, after several treatments tolerance developed. They concluded that gastric distention is not necessary in fenfluramine-induced anorexia, nor is tolerance to gastric emptying necessary in the development of tolerance to the anorexia. In addition, Neill and Cooper demonstrated that both D-fenfluramine (3 mg/kg, IP) and 5-HT (2 mg/kg, IP) reduced the intake of a sucrose solution in 4-h food-deprived, sham-feeding rats (13). These studies provide evidence that these drugs can produce an anorectic effect in the absence of an effect on gastric emptying, and suggest that the anorectic effects of 5-HT and fenfluramine may at best only partially depend on changes in the rate of gastric emptying.

The present data suggest that DL-fenfluramine may also affect food intake biphasically. At 0.1 mg/kg, DL-fenfluramine produced a small, albeit nonsignificant, increase in food intake compared to vehicle after 90 min. The effect of DL-fenfluramine did not conform to the dose-dependent decrease in food intake that normally would have been expected. This is interesting, because at the same dose DL-fenfluramine produced an increase in the rate of gastric emptying. Perhaps under some circumstances, an increase in gastric emptying may produce a hypherphagic effect. Such a result would strengthen the case that changes in gastric emptying affect food intake. However, any potential increase in food intake produced by low doses of DL-fenfluramine may be better observed in partially satiated rats consuming a palatable diet, a procedure that is more likely to reveal a hyperphagic effect.

In a comparison of the two drugs, therefore, the results indicate that 5-HT increased gastric emptying, but decreased food intake, within the same dose range, whereas DL-fenfluramine (1 and 3 mg/kg) produced similar effects on both measures. Taken together, these results suggest that the effects of 5-HT and DL-fenfluramine on feeding occur by different effects on the rate of gastric emptying. This is in part supported by Fletcher and Burton (7), who reported that peripherally administered 5-HT produced a greater suppression of food intake in vagotomized rats than in sham-operated controls. In contrast, D-fenfluramine produced a similar degree of anorexia in both groups of rats. They suggested that the receptor mechanism responsible for the anorectic action of 5-HT plays little or no part in the action of D-fenfluramine. Although their study was not directly concerned with the relationship between food intake and gastric emptying, the data indicate that under certain circumstances it is possible to observe a dissociation between the effects of 5-HT and DL-fenfluramine, as observed in the present study.

One conclusion from the present results is that the effects of 5-HT on food intake do not depend on changes in the rate of gastric emptying. However, this may be far too simplistic an explanation for what is obviously a complex phenomenon. DL-Fenfluramine produced decreases in both food intake and gastric emptying within the same dose range. This suggests that the effects of DL-fenfluramine on food intake may, to some extent at least, depend on changes in gastric emptying.

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