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5-HT and Carbohydrate Suppression: Effects of 5-HT Antagonists on the Action of *d*-Fenfluramine and DOI

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LAWTON, C. L. AND J. E. BLUNDELL. *5-HT and carbohydrate suppression: Effects of 5-HT antagonists on the action of* d-*fenfluramine and DOI.* PHARMACOL BIOCHEM BEHAV **46**(2) 349–360, 1993.—The effects of several 5-hydroxytryptamine (5-HT) receptor antagonists on the anorectic effect of *d*-fenfluramine and the 5-HT_2/5-HT_{1C} agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) were examined in a dietary paradigm that appears to be sensitive to 5-HT-induced carbohydrate suppression. In this paradigm, deprived rats are provided with a nutritionally complete hydrated chow mash diet together with an optional carbohydrate supplement of powdered Polycose. Both *d*-fenfluramine and DOI produced a clear suppression of total energy intake and carbohydrate (Polycose) intake. However, the mechanisms underlying these effects are different. The effect of *d*-fenfluramine in this paradigm was attenuated by the 5-HT_1/5-HT_2 receptor antagonist metergoline and partially attenuated by the 5-HT_{1A}/5-HT_{1B} receptor antagonist (±)cyanopindolol. In contrast, *d*-fenfluramine's effect was not antagonised by the 5-HT_2 receptor antagonist ketanserin, the 5-HT_3 receptor antagonist (3α-tropanyl)-1*H*-indole-3-carboxylic acid ester (ICS-205,930), the 5-HT_2/5-HT_{1C} receptor antagonist ritanserin, or the peripheral 5-HT receptor antagonist xylamidine. However, the effect of DOI in this paradigm was significantly attenuated by ketanserin but was not antagonised by either ritanserin or (±)cyanopindolol. Therefore, the suppressive effect of these two 5-HT drugs on total and Polycose intake appears to be mediated, respectively, by 5-HT_{1B}/5-HT_{1C} receptors (*d*-fenfluramine) and 5-HT_2 receptors (DOI).

Carbohydrate suppression 5-HT receptor subtypes *d*-Fenfluramine DOI Metergoline (±)Cyanopindolol Ketanserin ICS-205,930 Ritanserin Xylamidine

THE proposed existence of a biobehavioural feedback loop through which the selected composition of the diet modifies brain serotonin [5-hydoxytryptamine (5-HT)] synthesis and release and thus feeds back to influence the nutrients subsequently selected has generated considerable research interest. In particular, it has been argued that this control loop can regulate carbohydrate intake (33). This idea is, however, controversial and many animal studies have been conducted to test the proposed 5-HT/CHO link. While some studies yielded data in support of the hypothesis (18,20), others failed to do so (2,23,24,26). This issue has been the subject of a number of recent reviews (5,15).

The 5-HT anorectic agents *d*-fenfluramine (nonspecific) and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) [5-HT_2/5-HT_{1C} receptor agonist (12)] have both been shown to preferentially suppress carbohydrate intake in a dietary paradigm where deprived rats are presented with hydrated chow mash supplemented with powdered Polycose (16,17). This paradigm is an adaptation of one previously used by Sclafani and colleagues. In 1984, Sclafani and Xenakis (30) described an experimental procedure in which rats show an avid preference for sweet (sucrose) or bland (Polycose) carbohydrates presented as optional supplements to dry laboratory chow. We adopted this paradigm in the late 1980s as an alternative to traditional macronutrient selection paradigms as a new means of investigating drug effects on carbohydrate intake. After a long series of studies, we discovered that the effect of *d*-fenfluramine in this carbohydrate supplement model was influenced by the hydration of test diet components (16). Indeed, relative carbohydrate suppression was only observed when the chow was presented in hydrated form together with a dry carbohydrate supplement. Further, the effect was only demonstrated when Polycose, but not when sucrose, was used as the carbohydrate supplement.

This paradigm (hydrated chow/dry Polycose) provides a useful tool for further examination of 5-HT-induced anorexia. It also allows the investigation of the possible role of 5-HT receptor subtypes in the modulation of carbohydrate intake. The present studies, therefore, utilised this paradigm to investigate the receptor subtype(s) responsible for *d*-fenfluramine

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and DOI-induced changes in both total (chow plus Polycose) and Polycose intake.

Most of the research on fenfluramine indicates that 5-HT_1 receptors mediate fenfluramine and *d*-fenfluramine-induced anorexia (1,29). Further, because activation of 5-HT_{1A} receptors produces increases in food intake (7) it has generally been assumed that activation of 5-HT_{1B} and/or 5-HT_{1C} receptors is responsible for *d*-fenfluramine-induced anorexia. Research in this area has, however, been hindered by the lack of selective antagonists for 5-HT_{1B} and 5-HT_{1C} receptors (and 5-HT_{1D} receptors in humans).

In investigating the 5-HT receptor subtype(s) responsible for the action of *d*-fenfluramine in the present paradigm, several 5-HT receptor antagonists were employed in an attempt to block each subtype of the 5-HT receptor. The 5-HT antagonists used were: xylamidine, a peripheral 5-HT receptor antagonist (4) with some selectivity for 5-HT_2 receptors (10,21); ketanserin, which has a high affinity for 5-HT_2 sites and negligible affinity for 5-HT_1 sites (19); metergoline, a mixed 5-HT_1/5-HT_2 antagonist (25) with no affinity for 5-HT_3 receptor sites (3); ritanserin, a compound with high affinity for both 5-HT_2 and 5-HT_{1C} sites (13); (±)cyanopindolol, which has a high affinity for both 5-HT_{1A} and 5-HT_{1B} sites (8); and ICS-205,930 a selective 5-HT_3 receptor antagonist (27).

Schechter and Simansky (31) have already shown that the anorectic effect of DOI on a milk diet in rats was completely blocked by the 5-HT_2 receptor antagonists ketanserin and LY53587. The second experiment reported here, therefore, tests the hypothesis that the anorectic effect of DOI would be antagonised by ketanserin and ritanserin but not by (±)cyanopindolol. However, because DOI has activity at both 5-HT_2 and 5-HT_{1C} receptors and ritanserin has a higher affinity for 5-HT_2 receptors than ketanserin, while cyanopindolol has low 5-HT_{1C} affinity, it was acknowledged that distinguishing between these two receptor subtypes might be problematic.

METHOD

Animals

Fifty-six male, black hooded Lister rats (bred in our laboratories), in the weight range 303–419 g, were used. All animals were housed in individual cages in a quiet environment at constant temperature (20–22°C) with 20 complete air changes per hour. They were maintained on a 12 D : 12 L cycle with lights off at 0900 h. On nonexperimental days and subsequent to testing, animals were allowed ad lib access to laboratory chow in hydrated form. Water was freely available at all times. At the start of experiments, animals were divided into seven equal groups (n = 8) matched for body weight. Six groups were used in the *d*-fenfluramine study and the remaining group in the DOI study.

Drugs

The following drugs were either purchased from or gifts of the companies quoted in parentheses: *d*-fenfluramine HCl (Institut de Recherches Internationales Servier, Neuilly-sur-Seine, France), 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and ritanserin [Research Biochemicals, Inc.-Technical (UK) St. Albans, Herts, UK], xylamidine tosylate (The Wellcome Foundation, Beckenham, Kent, UK), ketanserin (Janssen Pharmaceutica, Beerse, Belgium), and (±)-cyanopindolol and (3α-tropanyl)-1*H*-indole-3-carboxylic acid ester (ICS-205,930) (Sandoz Products Ltd., Frimley, Camberley, Surrey, UK).

Both 5-HT agonists were dissolved in physiological saline and injected IP. The 5-HT receptor antagonists xylamidine and ICS-205,930 were dissolved in physiological saline. Ketanserin was dissolved in distilled water and metergoline was dissolved in 1.0% ascorbic acid (w/v) in distilled water. Ritanserin was dissolved in a vehicle of 20% propylene glycol in distilled water to which a few drops of lactic acid were added, followed by 10 N NaOH solution to bring vehicle plus drug to pH 5. (±)Cyanopindolol was dissolved in two to three drops of glacial acetic acid and made up to volume with physiological saline. Ketanserin, xylamidine, and metergoline were injected IP whereas ritanserin, ICS-205,930, and (±)cyanopindolol were injected SC.

All drug doses are expressed in terms of the salt or base as described. All drugs were injected in a volume of 1.0 ml/kg body weight with the exception of xylamidine, which was injected in a volume of 2.0 ml/kg body weight.

Diets

Laboratory chow (Labsure CRM (x)) in powdered form was mixed with water [37.7% chow (w/v), 5.12 kJ/g] and used as the maintenance diet. Powdered Polycose (Ross Laboratories, Columbus, OH, 16.00 kJ/g) was used as the dietary supplement. Both dietary commodities were presented in small Perspex pots.

Design

Six groups of animals were used in the *d*-fenfluramine study and were subdivided according to the 5-HT antagonist administered. Animals in these groups acted as their own controls across each of eight experimental treatments (vehicle/vehicle; low-dose antagonist/vehicle; middle-dose antagonist/vehicle; high-dose antagonist/vehicle; vehicle/*d*-fenfluramine; each dose of antagonist with *d*-fenfluramine). The remaining group of animals was used in the DOI study. Again, animals acted as their own controls across the experimental treatments administered [vehicle/vehicle; vehicle/DOI; ketanserin/DOI; ritanserin/DOI; (±)cyanopindolol/DOI].

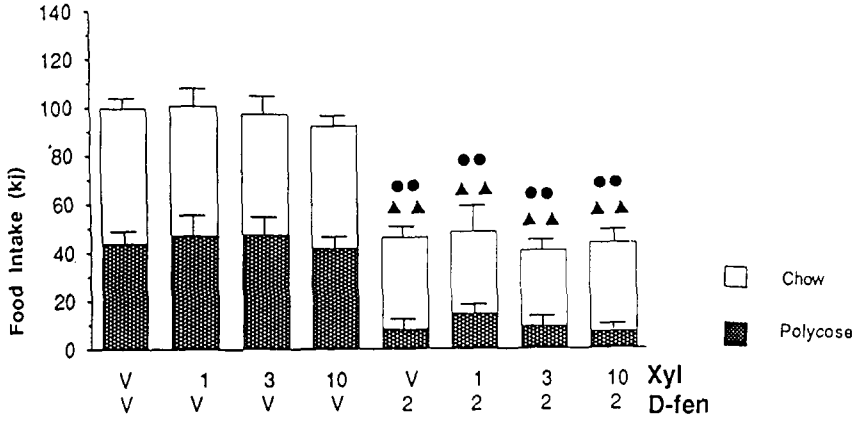
All treatments were administered in a counterbalanced order (Latin square) to minimise order effects. Further, drugs were administered blind, solutions being prepared and then independently coded prior to experimentation. A minimum period of 72 h separated successive treatments. Drug injections were staggered at 1-min intervals between animals, as were measurements of food intake. Hence, all consumption periods and times under drug influence were equal for all animals.

Procedure

During 3 weeks prior to the start of the studies, animals were acclimatised to all novel and possibly stressful features of the experiments. These included the reversed light/dark cycle, a 6-h food deprivation period, handling, drug injection procedures, test diets, and the experimental procedure. On each experimental day, the maintenance diet was removed from cages at the onset of darkness and the test diet (chow plus Polycose) was presented 6 h later. Animals received injections of the 5-HT antagonist used 1 h prior to food presentation with the exception of xylamidine, which was injected 3 h prior to food presentation. Both 5-HT agonists were injected 30 min prior to food presentation. Test diet components were presented in accurately weighed amounts. The amounts of each component remaining at 1 and 2 h were then measured

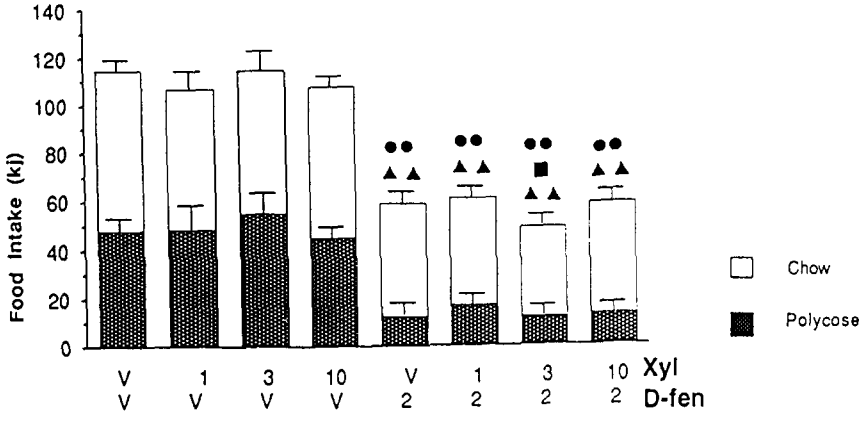
1 HOUR INTAKES

	% of total Intake							
Polycose	43.9	46.8	48.7	45.3	17.5	29.8	22.5	15.8
Xyl / D-fen	V/V	1/V	3/V	10/V	V/2	1/2	3/2	10/2



2 HOUR INTAKES

	% of total Intake							
Polycose	42.0	45.3	48.1	41.8	20.6	27.4	23.5	21.6
Xyl / D-fen	V/V	1/V	3/V	10/V	V/2	1/2	3/2	10/2

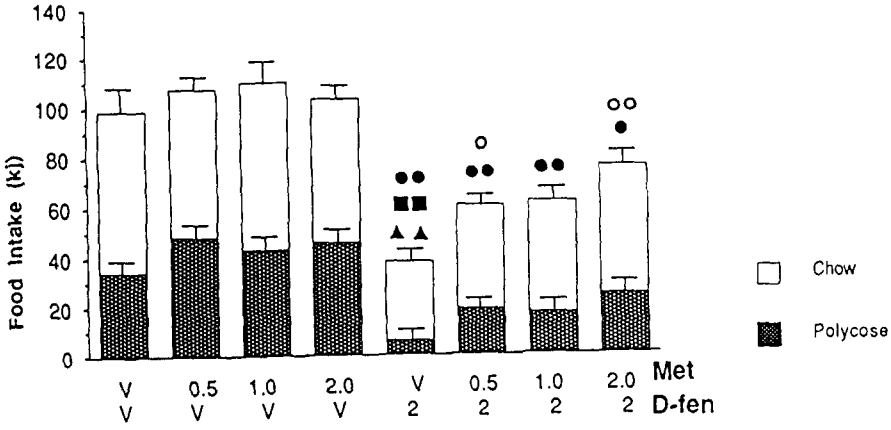


Dosage of Xylamidine / D-fenfluramine (mg/kg)

FIG. 1. Effect of 1.0, 3.0, and 10.0 mg/kg xylamidine on the anorectic effect of 2.0 mg/kg *d*-fenfluramine on mean (SE) intake of hydrated chow and powdered Polycose (kJ) during the 1- and 2-h periods following food presentation. Significant differences from control (v/v) values: (●), total intake; (■), chow intake; (▲), Polycose intake. Significant differences from *d*-fenfluramine (V/2): (○), total intake; (□), chow intake; (◇), Polycose intake. Single symbols indicate significance at $p < 0.05$ while double symbols indicate significance at $p < 0.01$.

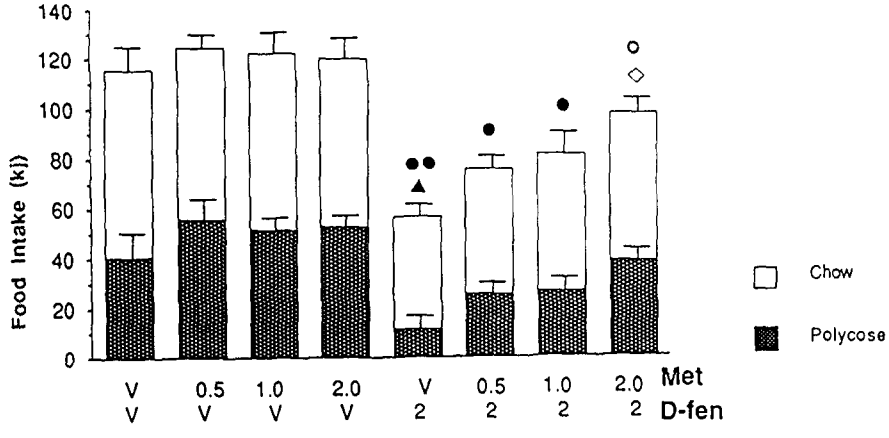
1 HOUR INTAKES

	% of total Intake							
Polycose	34.9	44.8	39.3	44.4	15.6	30.9	27.4	32.0
Met / D-fen	V/V	.5/V	1/V	2/V	V/2	.5/2	1/2	2/2



2 HOUR INTAKES

	% of total Intake							
Polycose	35.7	45.1	42.5	44.3	20.8	34.1	32.8	39.7
Met / D-fen	V/V	.5/V	1/V	2/V	V/2	.5/2	1/2	2/2

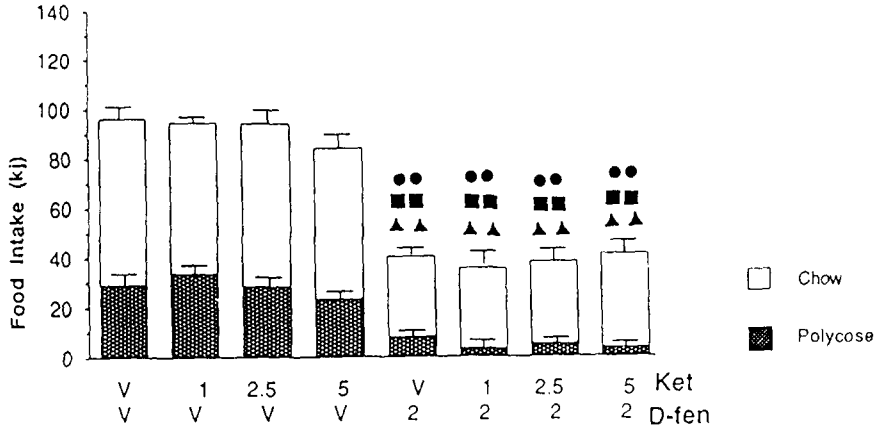


Dosage of Metergoline / D-fenfluramine (mg/kg)

FIG. 2. Effect of 0.5, 1.0, and 2.0 mg/kg metergoline on the anorectic effect of 2.0 mg/kg *d*-fenfluramine on mean (SE) intake of hydrated chow and powdered Polycose (kJ) during the 1- and 2-h periods following food presentation. All other details are as in Fig. 1.

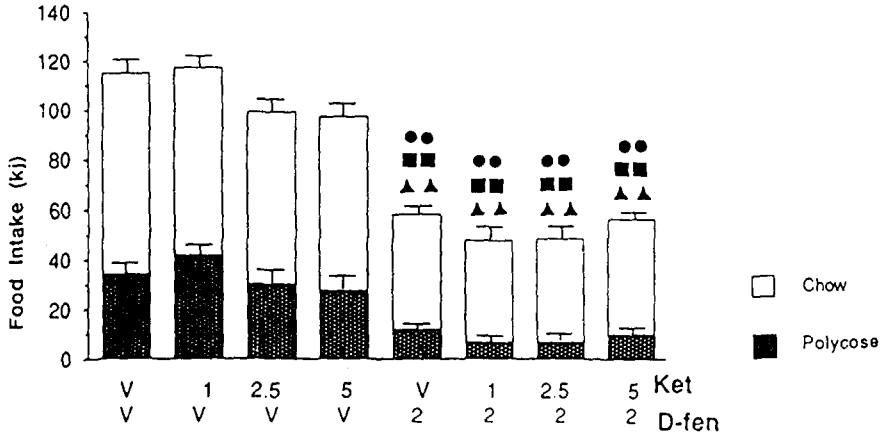
1 HOUR INTAKES

	% of total Intake							
Polycose	30.4	35.5	30.2	27.7	20.0	9.4	13.4	8.6
Ket / D-fen	V/V	1/V	2.5/V	5/V	V/2	1/2	2.5/2	5/2



2 HOUR INTAKES

	% of total Intake							
Polycose	29.3	35.3	30.3	28.0	20.7	14.0	13.9	17.8
Ket / D-fen	V/V	1/V	2.5/V	5/V	V/2	1/2	2.5/2	5/2

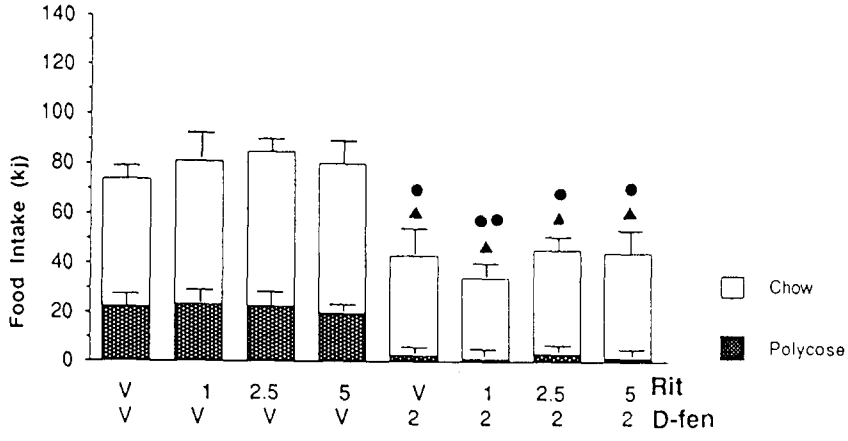


Dosage of Ketanserin / D-fenfluramine (mg/kg)

FIG. 3. Effect of 1.0, 2.5, and 5.0 mg/kg ketanserin on the anorectic effect of 2.0 mg/kg *d*-fenfluramine on mean (SE) intake of hydrated chow and powdered Polycose (kJ) during the 1- and 2-h periods following food presentation. All other details are as in Fig. 1.

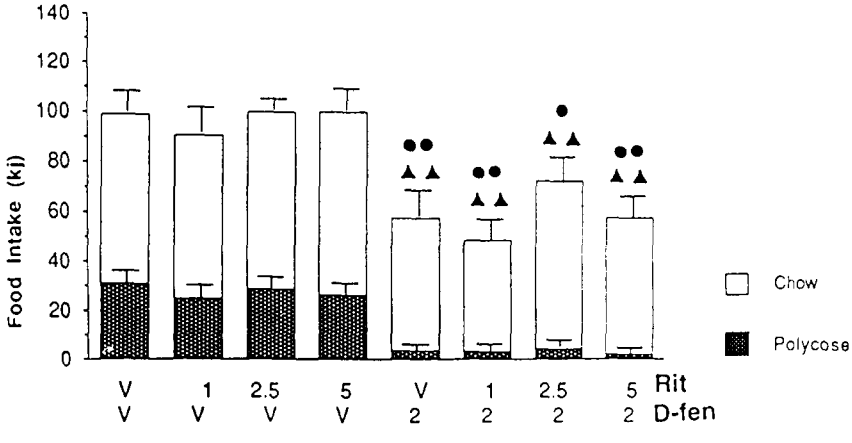
1 HOUR INTAKES

	% of total Intake							
Polycose	30.2	28.7	26.5	24.9	5.9	3.5	6.9	3.6
Rit / D-fen	V/V	.5/V	1/V	2/V	V/2	.5/2	1/2	2/2



2 HOUR INTAKES

	% of total Intake							
Polycose	31.2	27.5	28.5	26.3	6.2	6.5	5.8	3.4
Rit / D-fen	V/V	.5/V	1/V	2/V	V/2	.5/2	1/2	2/2

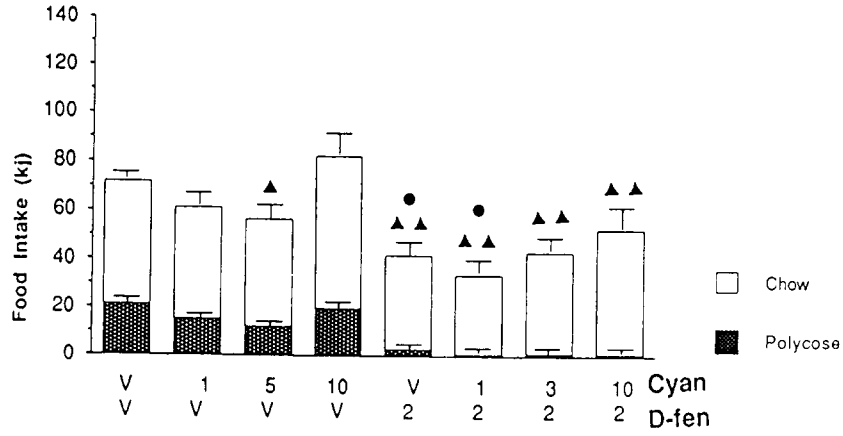


Dosage of Ritanserin / D-fenfluramine (mg/kg)

FIG. 4. Effect of 1.0, 2.5, and 5.0 mg/kg ritanserin on the anorectic effect of 2.0 mg/kg *d*-fenfluramine on mean (SE) intake of hydrated chow and powdered Polycose (kJ) during the 1- and 2-h periods following food presentation. All other details are as in Fig. 1.

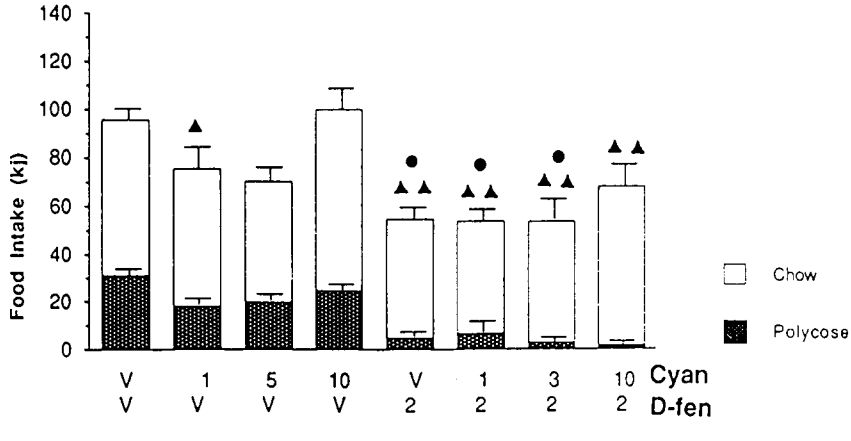
1 HOUR INTAKES

	% of total Intake							
Polycose	29.4	24.7	21.2	23.7	6.6	1.7	2.7	1.5
Cyan / D-fen	V/V	1/V	5/V	10/V	V/2	1/2	5/2	10/2



2 HOUR INTAKES

	% of total Intake							
Polycose	32.6	23.5	27.9	24.5	8.8	12.3	5.2	2.3
Cyan / D-fen	V/V	1/V	5/V	10/V	V/2	1/2	5/2	10/2

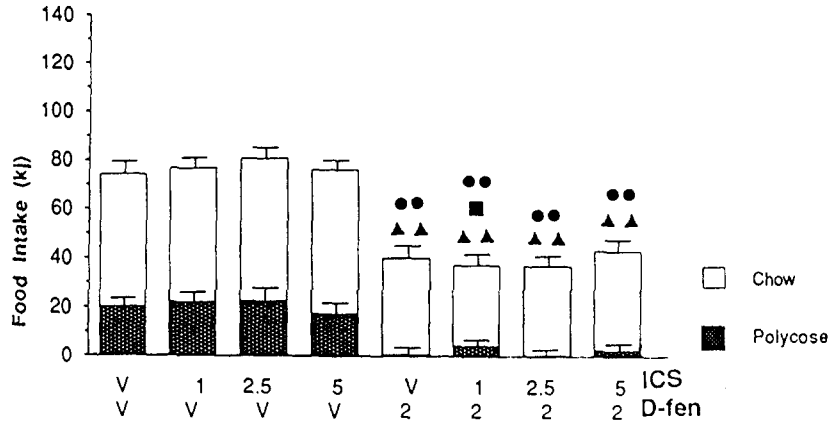


Dosage of Cyanopindolol / D-fenfluramine (mg/kg)

FIG. 5. Effect of 1.0, 5.0, and 10.0 mg/kg (±)cyanopindolol on the anorectic effect of 2.0 mg/kg *d*-fenfluramine on mean (SE) intake of hydrated chow and powdered Polycose (kJ) during the 1- and 2-h periods following food presentation. All other details are as in Fig. 1.

1 HOUR INTAKES

	% of total Intake							
Polycose	27.1	28.8	27.8	22.9	1.4	11.7	0.5	5.9
ICS / D-fen	V/V	1/V	2.5/V	5/V	V/2	1/2	2.5/2	5/2



2 HOUR INTAKES

	% of total Intake							
Polycose	27.90	28.60	26.63	21.15	5.78	8.15	3.66	9.50
ICS / D-fen	V/V	1/V	2.5/V	5/V	V/2	1/2	2.5/2	5/2

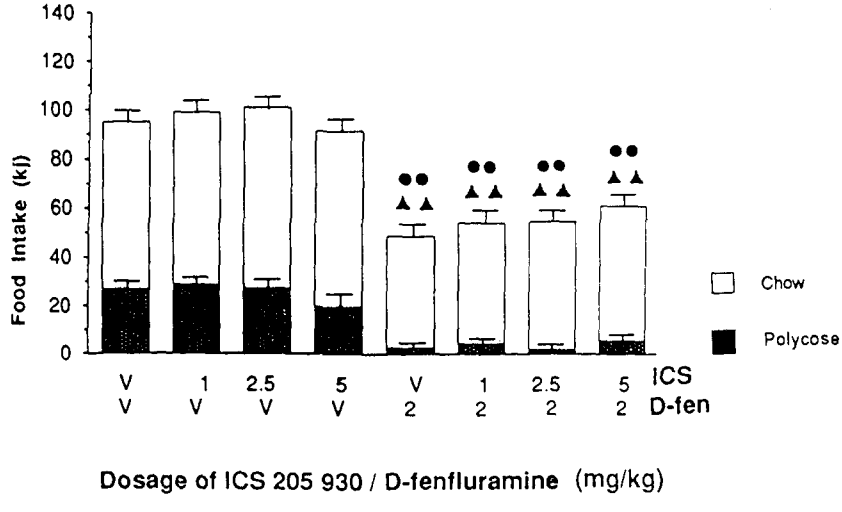


FIG. 6. Effect of 1.0, 2.5, and 5.0 mg/kg ICS-205,930 on the anorectic effect of 2.0 mg/kg *d*-fenfluramine on mean (SE) intake of hydrated chow and powdered Polycose (kJ) during the 1- and 2-h periods following food presentation. All other details are as in Fig. 1.

by successive weighing to the nearest 0.1 g. Care was taken to collect any food spillage and make the appropriate corrections.

Statistical Analysis

Data from each measurement interval (1 and 2 h) were analysed separately. In the *d*-fenfluramine study, data from each antagonist group were analysed separately. Total, chow, and Polycose intake data were analysed by two-way analyses of variance (ANOVAs) with two repeated measures (dose of *d*-fenfluramine and dose of antagonist). In the DOI study, total, chow, and Polycose intake data were analysed by one-way ANOVAs with one repeated measure (experimental treatment). Newman–Keuls a posteriori tests were used to detect significant differences between individual means.

RESULTS

d-Fenfluramine Study

The effects of xylamidine, metergoline, ketanserin, ritanserin, (±)cyanopindolol, and ICS-205,930 pretreatment on the anorectic effect of 2.0 mg/kg *d*-fenfluramine during the 1- and 2-h periods following food presentation are illustrated in Figs. 1–6, respectively.

Xylamidine/d-fenfluramine. During both time periods, xylamidine (1.0, 3.0, and 10.0 mg/kg) administered alone exerted no effect on total, absolute chow, or absolute Polycose intake. *d*-Fenfluramine administered alone, however, significantly decreased ($p < 0.01$) both total [1 h, $F(1, 7) = 154.68$, $p < 0.01$; 2 h, $F(1, 7) = 111.63$, $p < 0.01$] and absolute Polycose intake [1 h, $F(1, 7) = 50.48$, $p < 0.01$; 2 h, $F(1, 7) = 53.63$, $p < 0.01$] while leaving absolute chow intake relatively unaffected. This anorectic effect of *d*-fenfluramine was not antagonised by pretreatment with any of the doses of xylamidine used.

Metergoline/d-fenfluramine. During both time periods, there was a significant main effect of metergoline (0.5, 1.0, and 2.0 mg/kg) on absolute Polycose intake [1 h, $F(3, 21) = 3.25$, $p < 0.05$; 2 h, $F(3, 21) = 5.20$, $p < 0.01$]. Inspection of Fig. 2 indicates that this effect represents an overall increase in both absolute Polycose intake and in the percentage of total intake consumed as Polycose relative to baseline (vehicle/vehicle) values. This effect was selective for Polycose. No significant main effects of metergoline were apparent for total or absolute chow intake during these periods. *d*-Fenfluramine administered alone significantly decreased total, absolute chow, and absolute Polycose intake ($p < 0.01$) during the 1-h period [total, $F(1, 7) = 38.54$, $p < 0.01$; chow, $F(1, 7) = 24.67$, $p < 0.01$; Polycose, $F(1, 7) = 53.55$, $p < 0.01$] and decreased total ($p < 0.01$) and absolute Polycose intake ($p < 0.05$) during the 2-h period [total, $F(1, 7) = 92.17$, $p < 0.01$; Polycose, $F(1, 7) = 81.29$, $p < 0.01$]. *d*-Fenfluramine also strongly reduced the percentage of total intake consumed as Polycose relative to the baseline (vehicle/vehicle) values.

During both time periods, metergoline pretreatment exerted a tendency to reverse the anorectic effect of *d*-fenfluramine on absolute Polycose intake and consequently on total intake. Hence, metergoline acted to almost completely reverse the *d*-fenfluramine-induced reductions in the baseline percentage of total food intake consumed as Polycose. During the 1-h period, the inhibition of total intake observed with *d*-fenfluramine was significantly attenuated by 0.5-mg/kg ($p < 0.05$) and 2.0-mg/kg ($p < 0.01$) doses of metergoline. Further, during the 2-h period the inhibition of total and absolute Polycose intake observed with *d*-fenfluramine was significantly attenuated by the 2.0-mg/kg dose of metergoline ($p < 0.05$).

Ketanserin/d-fenfluramine. During both time periods, ketanserin (1.0, 2.5, and 10.0 mg/kg) administered alone exerted no effects on total, absolute chow, or absolute Polycose intake. *d*-Fenfluramine administered alone, however, significantly decreased ($p < 0.01$) total [1 h, $F(1, 7) = 160.92$, $p < 0.01$; 2 h, $F(1, 7) = 163.31$, $p < 0.01$], absolute chow [1 h, $F(1, 7) = 54.48$, $p < 0.01$; 2 h, $F(1, 7) = 153.81$, $p < 0.01$], and absolute Polycose intake [1 h, $F(1, 7) = 31.12$, $p < 0.01$; 2 h, $F(1, 7) = 35.33$, $p < 0.01$]. Additionally, *d*-fenfluramine reduced the baseline (vehicle/vehicle) percentage of total food intake consumed as Polycose. This anorectic effect of *d*-fenfluramine was not antagonised by any of the three doses of ketanserin used.

Ritanserin/d-fenfluramine. During the 1-h period, ritanserin (1.0, 2.5, and 5.0 mg/kg) administered alone exerted no significant effects on total, absolute chow, or absolute Polycose intake. During the 2-h period, however, analysis revealed a main effect of ritanserin on absolute Polycose intake that just reached significance at the $p = 0.05$ level, $F(3, 21) = 3.09$. Inspection of Fig. 4, however, indicates that this effect is difficult to interpret.

During both time periods, *d*-fenfluramine administered alone significantly reduced both total and absolute Polycose intake [$p < 0.05$, 1 h, total, $F(1, 7) = 52.22$, $p < 0.01$, Polycose, $F(1, 7) = 16.72$, $p < 0.01$; $p < 0.01$, 2 h, total, $F(1, 7) = 31.34$, $p < 0.01$, Polycose, $F(1, 7) = 16.86$, $p < 0.01$]. Absolute chow intake remained relatively unaffected. *d*-Fenfluramine, therefore, strongly reduced the percentage of total food intake consumed as Polycose relative to the baseline (vehicle/vehicle) values. The anorectic effect of *d*-fenfluramine on total and absolute Polycose intake was not significantly antagonised by any of the three doses of ritanserin used.

(±)Cyanopindolol/d-fenfluramine. During both time periods, (±)cyanopindolol (1.0, 3.0, and 10.0 mg/kg) exerted no significant effects on total or absolute chow intake. During the 1-h period only, however, there was a significant main effect of (±)cyanopindolol on absolute Polycose intake, $F(3, 21) = 3.65$, $p < 0.05$. Inspection of Fig. 5 reveals that the 5.0-mg/kg dose of (±)cyanopindolol significantly reduced absolute Polycose intake ($p < 0.05$). This effect was also observed with the 1.0-mg/kg dose during the 2-h period ($p < 0.05$).

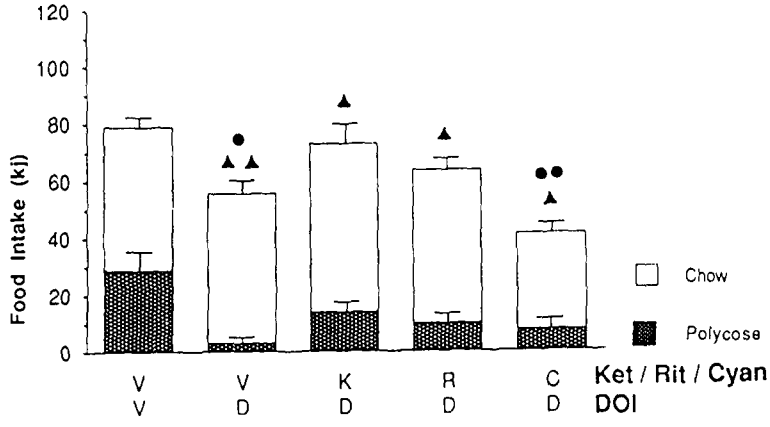
Administration of *d*-fenfluramine alone significantly decreased ($p < 0.05$) total intake [1 h, $F(1, 7) = 16.97$, $p < 0.01$; 2 h, $F(1, 7) = 8.20$, $p < 0.05$] and ($p < 0.01$) absolute Polycose intake [1 h, $F(1, 7) = 33.18$, $p < 0.01$; 2 h, $F(1, 7) = 22.17$, $p < 0.01$]. This anorectic effect of *d*-fenfluramine was not significantly antagonised by any of the three doses of (±)cyanopindolol used.

During both time periods, (±)cyanopindolol administered alone reduced the percentage of total intake consumed as Polycose relative to baseline (vehicle/vehicle) values. *d*-Fenfluramine, however, produced a much stronger reduction in this percentage. Interestingly, this reduction was potentiated by (±)cyanopindolol pretreatment.

ICS-205,930/d-fenfluramine. During both time periods, ICS-205,930 (1.0, 2.5, and 5.0 mg/kg) administered alone exerted no significant effects on total, absolute chow, or absolute Polycose intake. Administration of *d*-fenfluramine alone, however, significantly reduced ($p < 0.01$) total [1 h, $F(1, 7) = 43.16$, $p < 0.01$; 2 h, $F(1, 7) = 38.27$, $p < 0.01$] and absolute Polycose intake [1 h, $F(1, 7) = 21.87$, $p < 0.01$; 2 h,

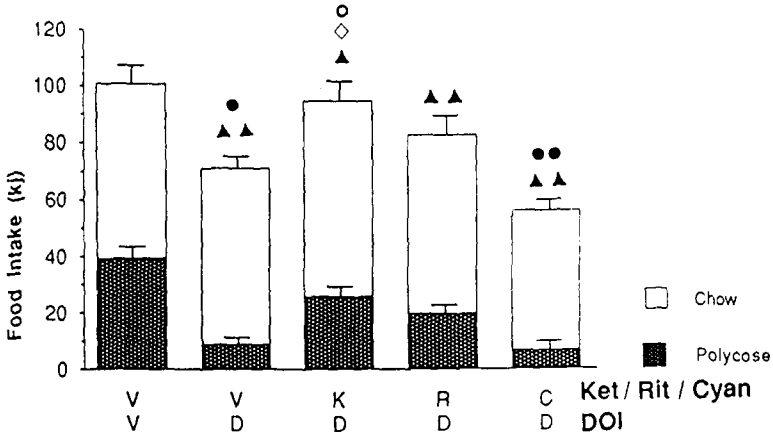
1 HOUR INTAKES

	% of total Intake				
Polycose	36.1	5.7	19.2	15.4	18.4
Treatment	V/V	V/D	K/D	R/D	C/D



2 HOUR INTAKES

	% of total Intake				
Polycose	39.0	12.6	27.2	23.9	11.7
Treatment	V/V	V/D	K/D	R/D	C/D



Dosage of DOI / Ketanserin / Ritanserin / Cyanopindolol

FIG. 7. Effect of 2.5 mg/kg ketanserin, 2.5 mg/kg ritanserin, and 5.0 mg/kg (±)cyanopindolol on the anorectic effect of 2.86 mg/kg 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) on mean (SE) intake of hydrated chow and powdered Polycose (kJ) during the 1- and 2-h periods following food presentation. Significant differences from control (v/v) values: (●), total intake; (■), chow intake; (▲), Polycose intake. Significant differences from DOI (V/D): (○), total intake; (□), chow intake; (◇), Polycose intake. Single symbols indicate significance at $p < 0.05$ while double symbols indicate significance at $p < 0.01$.

$F(1, 7) = 16.87, p < 0.01$] while leaving absolute chow intake relatively unaffected. This anorectic effect of *d*-fenfluramine was not antagonised by pretreatment with any of the doses of ICS-205,930 used.

DOI Study

The effects of 2.5 mg/kg ketanserin, 2.5 mg/kg ritanserin, and 5.0 mg/kg (±)cyanopindolol on the anorectic effect of 2.86 mg/kg DOI during the 1- and 2-h periods following food presentation are illustrated in Fig. 7.

Analysis revealed a main effect of treatment on total [1 h, $F(4, 28) = 7.84, p < 0.01$; 2 h, $F(4, 28) = 7.03, p < 0.01$] and absolute Polycose intake [1 h, $F(4, 28) = 3.96, p < 0.05$; 2 h, $F(4, 28) = 10.90, p < 0.01$] during both time periods. There was a main effect of treatment on absolute chow intake during the 1-h period only, $F(4, 28) = 4.63, p < 0.01$.

During both time periods, administration of DOI alone significantly reduced total ($p < 0.05$) and absolute Polycose intake ($p < 0.01$) while leaving absolute chow intake relatively unaffected. DOI, therefore, strongly reduced the baseline (vehicle/vehicle) percentage of total intake consumed as Polycose. During the 1-h period, the anorectic effect of DOI was not significantly attenuated by pretreatment with any of the three antagonists used. During the 2-h period, the anorectic effect of DOI was significantly attenuated by ketanserin only (total intake, $p < 0.05$).

DISCUSSION

The effects of *d*-fenfluramine administered alone in the present study confirm the findings of our previous studies (16,17). Hence, in all six groups of animals (subdivided according to antagonist administered) *d*-fenfluramine decreased total food intake (hydrated chow plus powdered Polycose) while also exerting a preferential suppression of Polycose intake. Further, the present results extend our previous findings because they demonstrate that *d*-fenfluramine-induced carbohydrate (Polycose) suppression is not restricted to the 1-h period following food presentation. These results, therefore, indicate that the suppression of Polycose induced by *d*-fenfluramine in this paradigm can be repeatedly demonstrated under appropriate experimental circumstances.

The effects of DOI administered alone in the same paradigm also confirm the results obtained with this drug in a previous experiment (17). Hence, DOI produced almost equivalent effects to those observed with *d*-fenfluramine. Together, these findings confirm the sensitivity of the chosen dietary paradigm to 5-HT-induced carbohydrate (Polycose) suppression.

Both metergoline and (±)cyanopindolol exerted significant effects on Polycose intake when administered alone. The small increases in Polycose intake found with metergoline in the present study are consistent with the increases in food intake (6,9) and carbohydrate preference (32) found with this antagonist in other feeding situations. It is not clear, however, why (±)cyanopindolol should decrease Polycose intake. Xylamidine, ketanserin, and ICS-205,930 did not exert any significant effects on food intake when administered alone. A main effect of ritanserin on chow intake was revealed from analysis of 2-h food intake data. This significant main effect is, however, difficult to interpret.

The lack of antagonism shown by xylamidine indicates that central, rather than peripheral, 5-HT receptors were involved in the action of *d*-fenfluramine to inhibit food intake and decrease the percentage of total intake consumed as Polycose. The effect of *d*-fenfluramine in this paradigm does not, therefore, appear to be dependent upon any peripheral effect of the drug such as an inhibition of gastric emptying.

The anorectic effect of *d*-fenfluramine in this test situation was, however, attenuated by metergoline but not by ketanserin or ICS-205,930. The effects of metergoline, ketanserin, and ICS-205,930 on the anorectic effect of *d*-fenfluramine together suggest that the effect of metergoline was due to its ability to act as an antagonist at 5-HT_1 receptors. Support for this hypothesis comes from the finding that metergoline antagonises the anorectic effect of 5-HT_1 receptor agonists (1,14,28). The present data, therefore, implicate 5-HT_1 but not 5-HT_2 or 5-HT_3 receptors in the mediation of the anorectic effect of *d*-fenfluramine at least in this dietary choice situation.

The inability of ritanserin to antagonise the anorectic effect of *d*-fenfluramine in this paradigm is consistent with the results of Garattini et al. (11) but inconsistent with the results of Neill and Cooper (22). The effects of ketanserin and ritanserin pretreatment on the anorectic effect of *d*-fenfluramine together, therefore, appear to rule out a selective role of 5-HT_{1C} receptors in mediating this effect.

The ability of (±)cyanopindolol to weakly antagonise the anorectic effect of *d*-fenfluramine in this paradigm is consistent with the results of Neill and Cooper (22). Tentative evidence for a role of 5-HT_{1B} receptors was suggested because during both the 1- and 2-h periods following food presentation 10.0 mg/kg (±)cyanopindolol (which had no effects on food intake when administered alone) showed a nonsignificant tendency to attenuate the anorectic effect of *d*-fenfluramine on total intake.

Considering the actions of these different 5-HT antagonists together with the knowledge that 5-HT_{1A} receptor activation produces increases in food intake, our understanding regarding the action of *d*-fenfluramine is summarised as follows; The anorectic effect of *d*-fenfluramine in the present paradigm was blocked by metergoline but not by ketanserin or ICS-205,930. Therefore, we infer that the action of *d*-fenfluramine is mediated by 5-HT_1 and not by 5-HT_2 or 5-HT_3 receptors. However, because neither ritanserin (fairly selective for 5-HT_{1C} receptors) nor (±)cyanopindolol (fairly selective for 5-HT_{1B} receptors) significantly antagonised the anorectic effect of *d*-fenfluramine we infer that the separate blockade of these receptors is insufficient to bring about such an effect. From this, we make the cautious deduction that the *d*-fenfluramine suppressive effect is due to a joint action at 5-HT_{1B} and 5-HT_{1C} receptors.

Although 5-HT_2 receptors do not appear to be importantly involved in the anorectic effect of *d*-fenfluramine in this paradigm, this does not rule out a role for 5-HT_2 receptors in either appetite or carbohydrate suppression. Indeed, the 5-HT_2 receptor agonist DOI had essentially the same anorectic effect as *d*-fenfluramine in the present paradigm. Additionally, this anorectic effect of DOI was antagonised by ketanserin and not by (±)cyanopindolol. Further, ritanserin displayed a nonsignificant marginal attenuation of the anorectic effect of DOI. The antagonism of the anorectic effect of DOI in the present paradigm and on a milk diet (31) give some support to the idea that the anorectic effect of DOI is mediated by 5-HT_2 receptors. However, the antagonism of DOI by ketanserin and ritanserin in this paradigm is not clearly defined and hence it is necessary to be cautious about the diagnosis of the receptor activity underlying these actions. Additionally, because DOI also exerts an action at 5-HT_{1C} receptors further work is required to determine the importance of the

role of 5-HT_2 receptors in appetite and carbohydrate suppression.

The results of the present studies suggest that activation of 5-HT_1 (probably 5-HT_{1B} and 5-HT_{1C}) and 5-HT_2 receptors alone, by *d*-fenfluramine and DOI, respectively, is sufficient to cause an inhibition of total food intake and a selective suppression of carbohydrate intake, at least when rats are offered powdered Polycose as an optional supplement to hydrated chow. In conclusion, although *d*-fenfluramine and DOI produced similar changes in consumption patterns within this dietary paradigm these effects are clearly due to the operation of separate 5-HT receptor subtypes.

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REFERENCES

1. Bendotti, C.; Samanin, R. The role of putative 5-HT_{1A} and 5-HT_{1B} receptors in the control of feeding in rats. Life Sci. 41:635–642; 1987.
2. Blundell, J. E.; Hill, A. J. Do serotoninergic drugs decrease energy intake by reducing fat or carbohydrate intake? Effects of *d*-fenfluramine with supplemented weight-reducing diets. Pharmacol. Biochem. Behav. 31:773–778; 1989.
3. Bradley, P. B.; Engel, G.; Fenuik, W.; Fozard, J. R.; Humphrey, P. P. A.; Middlemiss, D. N.; Mylecharane, E. J.; Richardson, B. P.; Saxena, P. R. Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology 25:563–576; 1986.
4. Copp, F. C.; Green, A. F.; Hodson, H. F.; Randall, A. W.; Sim, M. F. New peripheral antagonists of 5-hydroxytryptamine. Nature 214:200–201; 1967.
5. Dourish, C. T. 5-HT receptor subtypes and feeding behaviour. In: Bradley, P. B.; Handley, S. L.; Cooper, S. J.; Key, B. J.; Barnes, N. M.; Coote, J. H., eds. Serotonin, CNS receptors and brain function. Advances in the biosciences. vol. 85. Oxford, UK: Pergamon Press Ltd.; 1992:179–202.
6. Dourish, C. T.; Clark, M. L.; Fletcher, A.; Iverson, S. D. Evidence that blockade of postsynaptic 5-HT_1 receptors elicits feeding in satiated rats. Psychopharmacology (Berl.) 97:54–58; 1989.
7. Dourish, C. T.; Hutson, P. H.; Curzon, G. Low doses of the putative serotonin agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) elicit feeding in the rat. Psychopharmacology (Berl.) 86:197–204; 1985.
8. Engel, G.; Gothert, M.; Hoyer, D.; Schlicker, E.; Hillenbrand, K. Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. Naunyn Schmiedeberg Arch. Pharmacol. 332:1–7; 1986.
9. Fletcher, P. J. Increased food intake in satiated rats induced by 5-HT antagonists methysergide, metergoline and ritanserin. Psychopharmacology (Berl.) 96:237–242; 1988.
10. Fuller, R. W.; Kurz, K. D.; Mason, N. R.; Cohen, M. L. Antagonism of a peripheral vascular but not an apparently central serotonergic response by xylamidine and BW 501C 67. Eur. J. Pharmacol. 125:71–77; 1986.
11. Garattini, S.; Mennini, T.; Samanin, R. From fenfluramine racemate to *d*-fenfluramine: Specificity and potency of the effects on the serotoninergic system and food intake. Ann. NY Acad. Sci. 499:156–166; 1986.
12. Glennon, R. A. Discriminative properties of the serotoninergic agent 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Life Sci. 39:825–830; 1986.
13. Hoyer, D. Functional correlates of serotonin 5-HT_{1C} recognition sites. J. Receptor Res. 8:59–81; 1988.
14. Kennett, G. A.; Dourish, C. T.; Curzon, G. 5-HT_{1B} agonists induce anorexia at a postsynaptic site. Eur. J. Pharmacol. 141: 429–435; 1987.
15. Lawton, C. L. Investigation of the action of serotoninergic drugs on carbohydrate intake in the rat. Ph.D. thesis, University of Leeds, Leeds, UK, 1991.
16. Lawton, C. L.; Blundell, J. E. The effect of *d*-fenfluramine on intake of carbohydrate supplements is influenced by the hydration of test diets. Behav. Pharmacol. 3:517–523; 1992.
17. Lawton, C. L.; Blundell, J. E. 5-HT manipulation and dietary choice: Variable carbohydrate (Polycose) suppression demonstrated only under specific experimental conditions. Psychopharmacology; in press.
18. Leibowitz, S. F.; Weiss, G. F.; Walsh, U. A.; Viswanath, D. Medial hypothalamic serotonin: Role in circadian patterns of feeding and macronutrient selection. Brain Res. 503:132–140; 1989.
19. Leyson, J. E.; Awouters, F.; Kennis, L.; Laduron, P. M.; Vanderburk, J.; Janssen, P. A. J. Receptor binding profile of R41 468, a novel antagonist at 5-HT_2 receptors. Life Sci. 28:1015–1022; 1981.
20. Luo, S.; Li, E. T. S. Food intake and selection pattern of rats treated with dexfenfluramine, fluoxetine and RU24969. Brain Res. Bull. 24:729–733; 1990.
21. Middlemiss, D. N.; Hibert, M.; Fozard, J. R. Drugs acting at central 5-hydroxytryptamine receptors. Annu. Rep. Med. Chem. 21:41–50; 1986.
22. Neill, J. C.; Cooper, S. J. Evidence that *d*-fenfluramine anorexia is mediated by 5-HT_1 receptors. Psychopharmacology (Berl.) 97: 213–218; 1989.
23. Orthen-Gambill, N. Sucrose intake unaffected by fenfluramine but suppressed by amphetamine administration. Psychopharmacology (Berl.) 72:130–135; 1985.
24. Orthen-Gambill, N.; Kanarek, R. B. Effects of amphetamine and fenfluramine on dietary self-selection in rats. Pharmacol. Biochem. Behav. 16:303–309; 1982.
25. Peroutka, S. J.; Lebovitz, R. M.; Snyder, S. H. Two distinct central serotonin receptors with different physiological functions. Science 212:827–829; 1981.
26. Peters, J. C.; Bellissimo, D. B.; Harper, A. E. L-Tryptophan injection fails to alter nutrient selection by rats. Physiol. Behav. 32:253–259; 1984.
27. Richardson, B. P.; Engel, G.; Donatsch, P.; Stadler, P. A. Identification of 5-hydroxytryptamine M-receptor subtypes and their specific blockade by a new class of drugs. Nature 316:126–131; 1985.
28. Samanin, R.; Bendotti, C.; Candalares, G.; Garattini, S. Specificity of serotonergic involvement in the decrease of food intake induced by quipazine in the rat. Life Sci. 21:1259–1266; 1977.
29. Samanin, R.; Garattini, S. Serotonin and the pharmacology of eating disorders. Ann. NY Acad. Sci. 575:194–208, 1989.
30. Sclafani, A.; Xenakis, S. Sucrose and polysaccharide induced obesity in the rat. Physiol. Behav. 32:169–174; 1984.
31. Schechter, L. E.; Simansky, K. J. 1-(2,5-dimethoxy-4-iodophenyl)-2-Aminopropane (DOI) exerts an anorexic action that is blocked by 5-HT_2 antagonists in rats. Psychopharmacology (Berl.) 94:342–346; 1988.
32. Stallone, D.; Nicholaidis, S. Increased food intake and carbohydrate preference in the rat following treatment with the serotonin antagonist metergoline. Neurosci. Lett. 102:319–324; 1989.
33. Wurtman, J. J.; Wurtman, R. J. Drugs that enhance central serotoninergic transmission diminish elective carbohydrate consumption by rats. Life Sci. 24:895–904; 1979.