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ORIGINAL INVESTIGATION

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Behavioural evidence that *d*-fenfluramine-induced anorexia in the rat is not mediated by the 5-HT_{1A} receptor subtype

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Abstract These studies investigated the involvement of the 5-HT_{1A} receptor in mediating *d*-fenfluramine-induced anorexia in the rat. Non-deprived, *d*-fenfluramine-treated (3.0 mg/kg) rats consumed a reduced amount of a palatable wet mash and showed a temporal advance in the behavioural sequence consistent with satiety. Thus, rats treated with *d*-fenfluramine ceased feeding and began resting before corresponding controls. Pretreatment with the selective 5-HT_{1A} receptor antagonist WAY-100635 (1.0 mg/kg) had no effect on either the reduced mash consumption or behavioural satiety sequence of *d*-fenfluramine-treated animals at a dose which was found to attenuate the anorexia induced by the 5-HT_{1A} receptor agonist 8-OH-DPAT (0.5 mg/kg). Pretreatment with the non-selective 5-HT antagonist metergoline (1.0 mg/kg) attenuated the *d*-fenfluramine-induced reduction of mash consumption and the advanced offset of feeding. Metergoline pretreatment had no effect on the advanced onset of resting observed in *d*-fenfluramine-treated animals. These data suggest that *d*-fenfluramine reduces food intake, perhaps by enhancing satiety, via a mechanism which does not involve the 5-HT_{1A} receptor subtype. The implications of these results to the utility of the behavioural satiety sequence as a measure of postprandial satiety are discussed.

Key words *d*-Fenfluramine · Metergoline · 8-OH-DPAT · WAY-100635 · Anorexia · Satiety sequence · Rat

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Introduction

Fenfluramine releases 5-hydroxytryptamine (5-HT; serotonin) from presynaptic terminals and inhibits its re-uptake (Garattini et al. 1986; Carboni and Di Chiara 1989). Although the serotonergic basis of fenfluramine-induced hypophagia has been questioned (Gibson et al. 1993), considerable evidence exists to suggest that the stimulatory effect of fenfluramine on the serotonergic system is linked to its anorectic action in animals and humans (for reviews see Blundell 1984; Rowland and Carlton 1986; Dourish 1992).

Early studies suggested that the anorexia induced by racemic fenfluramine, or by its more potent *d*-isomer (Mennini et al. 1985), could be attenuated by non-specific 5-HT antagonists such as metergoline (Blundell and Latham 1980a; Garattini et al. 1986; Goodall and Silverstone 1988), methysergide (Barrett and McSharry 1975) and the 5-$HT_{1A/1B}$/β-adrenoceptor antagonist (±)-cyanopindolol (Neill and Cooper 1989; Grignaschi and Samanin 1992). In contrast to these findings, *d*-fenfluramine-induced anorexia was not blocked by the 5-HT_3 antagonist ICS 205 930 (Neill and Cooper 1989), or the peripheral 5-HT receptor antagonist xylamidine (Borsini et al. 1985; Neill and Cooper 1989). Studies investigating the role of the 5-HT_2 receptor subtype in mediating *d*-fenfluramine-induced anorexia have yielded conflicting results. The anorectic effect of *dl*-fenfluramine has been reported to be antagonised by the 5-HT_{2A} antagonist ketanserin (Hewson et al. 1988) and, in the case of the *d*-isomer, by the 5-$HT_{2A/2C}$ antagonist ritanserin (Neill and Cooper 1989; Goodall et al. 1993). However, there are reports of ritanserin failing to attenuate *d*-fenfluramine-induced anorexia (Samanin et al. 1989; Grignaschi and Samanin 1992)

Thus, studies with non-specific antagonists suggest that the 5-HT_{1A}, 5-HT_{1B} and possibly the 5-HT_{2C} receptor subtypes may be of particular importance in mediating *d*-fenfluramine-induced anorexia (Neill and Cooper 1989; Samanin et al. 1989; Grignaschi and

Samanin 1992). The present experiments attempt to elucidate the role of the 5-HT_{1A} receptor subtype in mediating the anorectic effects of *d*-fenfluramine by using a novel, selective, 5-HT_{1A} antagonist.

When allowed to feed to satiety, rats exhibit a characteristic sequence of behaviours (Antin et al. 1975). Thus, the cessation of feeding is superseded by a brief period of active behaviours, including locomotion, rearing and grooming, which is in turn followed by a longer period of rest and/or sleep. This postprandial sequence of behaviour has been characterised as the behavioural satiety sequence (Antin et al. 1975) and has been used to discriminate between the anorexia induced by manipulations which potentiate endogenous satiation and satiety, and the reduction in feeding caused by the induction of sickness or stimulant nature of the treatment (Antin et al. 1975; Kitchener and Dourish 1994).

The current studies investigate the effect of pretreatment with the mixed 5-$HT_{1/2}$ antagonist metergoline (Hoyer 1988) and the silent, selective, 5-HT_{1A} antagonist WAY-100635 (Fletcher et al. 1995; Forster et al. 1995) on changes in the behavioural satiety sequence induced by *d*-fenfluramine administration. The doses of *d*-fenfluramine (3.0 mg/kg) and metergoline (1.0 mg/kg) chosen have previously been reported to reduce food intake in a test meal by half and antagonise this effect, respectively (Neill and Cooper 1989).

5-HT_{1A} receptors are localised both pre- and postsynaptically (for reviews see Palacios et al. 1987; Fletcher et al. 1993). The activation of postsynaptic 5-HT_{1A} receptors leads to the induction of a characteristic behavioural syndrome (Tricklebank et al. 1984). Thus, doses of 8-OH-DPAT that induce this syndrome also decrease food intake in hungry rats and this has been suggested to be a non-specific hypophagia induced by response competition (Dourish et al. 1985b).

We report dose-response data for the attenuation of 8-OH-DPAT-induced (0.5 mg/kg) hypophagia by WAY-100635. The dose of WAY-100635 (1.0 mg/kg) subsequently chosen to challenge the reduction in food intake induced by *d*-fenfluramine treatment completely blocked 8-OH-DPAT-induced hypophagia. Although this dose of WAY-100635 has a high 5-HT_{1A} receptor occupancy, it has no intrinsic effect in several models of pre- and postsynaptic 5-HT_{1A} receptor activation (Fletcher et al. 1995). Thus, the risk of this dose of WAY-100635 evoking non-specific effects is minimal.

Materials and methods

Animals

Subjects were 80 male, hooded Lister rats (University of Sussex colony), weighing in the range of 280–350 g at the onset of the experiments. Animals were singly housed with free access to standard laboratory chow (B&K Universal Group Ltd, Hull, UK) and tap water. Experimental rooms were maintained at 21–22°C on a 12-h light: 12-h dark cycle (lights on 0800 hours). A red light was the sole source of illumination during the dark period. The work reported in this manuscript was performed in accordance with Home Office regulations as outlined in the Animals (Scientific Procedures) Act 1986.

Drugs

d-Fenfluramine hydrochloride, generously supplied by Institut de Recherches Internationales Servier, Neuilly-sur-Seine, France, was dissolved in 0.9% saline. Metergoline (Research Biochemicals) was ultrasonically dispersed in 1% ascorbic acid. Both *d*-fenfluramine and metergoline were administered via the intraperitoneal route. 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) and WAY-100635(N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide trihyrochloride), supplied by Wyeth Research (UK) Ltd, were dissolved in 0.9% saline and administered subcutaneously at the nape of the neck. All drug administrations were in the volume 1.0 ml/kg and the appropriate volumes of vehicle were used as controls.

Test diet

For the duration of all experiments animals were presented daily with a wet mash meal (made by soaking 500 g of crushed diet with 500 ml of tap water before mixing to a smooth consistency) in preweighed clear glass containers. Consumption was recorded to the nearest 0.1 g and care was taken to collect any spillage and make appropriate corrections.

Procedure

Experiment 1: effects of WAY-100635 on d-fenfluramine-induced changes in the behavioural satiety sequence

Twelve rats were used in this study. All testing was carried out in the home cage and test sessions were 40 min in duration. Animals were presented with the test meal over a period of 8 days in the absence of any experimental observations. On the final day of this habituation period all animals were injected with 0.9% saline and their behaviour observed for a period of 40 min. Hence, prior to receiving any drug treatments all animals had been acquainted with the experimental procedure.

Behavioural observations were performed as previously reported (Clifton et al. 1989). A microprocessor was programmed to illuminate individually a small LED adjacent to each cage every 2.5 s. As a LED lit up, the observer pressed the key on a hand-held keypad corresponding to the behaviour which the indicated animal exhibited. The key press turned the LED off and 2.5 s later the LED beside the next cage was lit; this process was repeated for the 40-min test period. Hence, each animal was observed every 30 s and 80 times in total for each session. Four mutually exclusive categories were used to record behaviour; feeding (including acquisition and chewing of the food substrate and the rare occasions where animals took a drink), active (encompassing locomotion, rearing and sniffing, and was used when no other category was deemed appropriate), grooming (face washing and repetitive licks directed to the body) and resting (adoption of a prone position with or without eyes closed).

On each experimental day, WAY-100635 (vehicle or 1.0 mg/kg) was injected 30 min prior to *d*-fenfluramine (vehicle or 3.0 mg/kg). The wet mash test meal was presented 30 min after *d*-fenfluramine (or vehicle) treatment and behavioural observation subsequently

commenced. Each animal acted as its own control and received each of the four combinations of treatment in a counterbalanced order. A period of 48 h was left between successive experimental days.

Experiment 2: effects of metergoline on d-fenfluramine-induced changes in the behavioural satiety sequence

Twelve rats were used in this study and behavioural procedures for habituation and testing were identical to those described for experiment 1. On each experimental day metergoline (vehicle or 1.0 mg/kg) was administered 30 min prior to *d*-fenfluramine (vehicle or 3.0 mg/kg). Testing began 30 min after *d*-fenfluramine treatment. Each animal acted as its own control and received each of the four combinations of treatment in a counterbalanced order. Experimental days were separated by 48 h.

Experiment 3: attenuation of 8-OH-DPAT-induced hypophagia by WAY-100635

Fifty-six rats were used in this study. The experimental protocol was identical to that described for the previous experiments with a distinction in that no behavioural observation was undertaken on these animals. WAY-100635 (saline, 0.003, 0.01, 0.03, 0.1, 0.3, or 1.0 mg/kg) was administered 30 min prior to 8-OH-DPAT (saline or 0.5 mg/kg) injection. Testing began 30 min after 8-OH-DPAT treatment. Each animal received vehicle and 8-OH-DPAT and was assigned to one particular dose of WAY-100635. One week was allowed between the 2 experimental days.

Statistics

The mash intake data from experiments 1 and 2 were subject to two-way analyses of variances. Comparisons between individual treatment group means and a control mean were performed using Dunnett's test. Appropriate comparisons between treatment means at the same dose of *d*-fenfluramine were performed using *t*-test with Bonferroni correction. Behavioural observations were summed into 5-min blocks and subjected to three-way analysis of variance supplemented by planned comparisons. In these analyses both antagonist and drug dose were always within-subjects factors. One animal in experiment 2 failed to habituate to behavioural observations being taken during the test meal and was eliminated from all analysis. The analyses of variances performed for this experiment utilised the missing values procedures of the GENSTAT statistical package. Hence, one fewer degree of freedom in the numerator than would be expected from the experimental design is reported.

Experiment 3 is a two-way mixed design. The intake data from this experiment were subject to a two-way analysis of variance where WAY-100635 dose was a between-subject factor and 8-OH-DPAT dose was a within-subject factor. A significant interaction was followed up by performing both Dunnett's tests to compare treatment means against the control mean and Holm's multistage Bonferroni procedure (Howell 1992) to compare vehicle and 8-OH-DPAT treatments at each dose of WAY-100635.

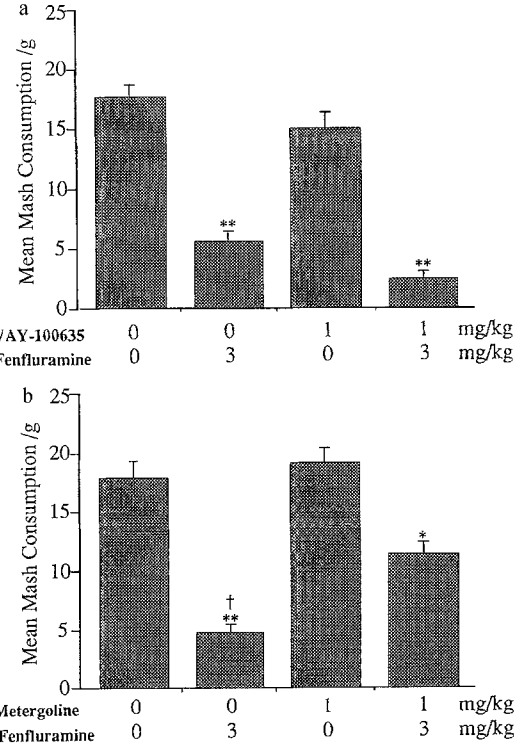
Results

Experiment 1: the effects of WAY-100635 on *d*-fenfluramine-induced changes in the behavioural satiety sequence

Ingestion of wet mash during the observation period led to the development of a typical satiety sequence in control animals (Fig. 2, top left panel). *d*-Fenfluramine-treatment significantly reduced wet mash intake in the 40-min test period [$F(1, 11) = 112.57$, $P < 0.001$]. Pretreatment with the 5-HT_{1A} antagonist WAY-100635 failed to block the *d*-fenfluramine-induced reduction in mash intake [Fig. 1a: $F(1, 11) = 0.004$, n.s.].

d-Fenfluramine treatment significantly reduced the proportion of time spent feeding especially in early time bins and this led to a significant interaction between time and drug treatment [$F(7, 77) = 5.00$, $P < 0.001$)]. WAY-100635 had no effect on this pattern [drug × antag $F(1, 11) = 3.81$, n.s. ; antag × time × drug $F(7, 77) = 1.46$, n.s.]. *d*-Fenfluramine significantly increased the frequency of active behaviours [$F(1, 11) = 9.19$, $P < 0.05$] and led to a significant time × drug interaction [$F(7, 77) = 5.99$, $P < 0.001$]. WAY-100635 pretreatment failed to reverse either of these effects. During the initial time bins, *d*-fenfluramine significantly reduced the incidence of feeding as compared to vehicle-treated animals yet had no significant effect on the incidence of active behaviours (Fig. 2, bottom left panel). Such results suggest that the early offset of feeding cannot be attributed exclusively to increased activity but rather,

Fig. 1 **a** The effect of WAY-100635 administration on wet mash intake over a 40-min period following *d*-fenfluramine treatment ($n = 12$). * $P < 0.05$, ** $P < 0.01$, compared to vehicle/vehicle condition. WAY-100635 was injected 30 min prior to drug treatment. **b** The effect of metergoline administration on wet mash intake over a 40-min period following *d*-fenfluramine treatment ($n = 11$). * $P < 0.05$, ** $P < 0.01$, compared to vehicle/vehicle condition. † $P < 0.05$, comparison between treatment means at the same dose of *d*-fenfluramine. Metergoline was injected 30-min prior to drug treatment



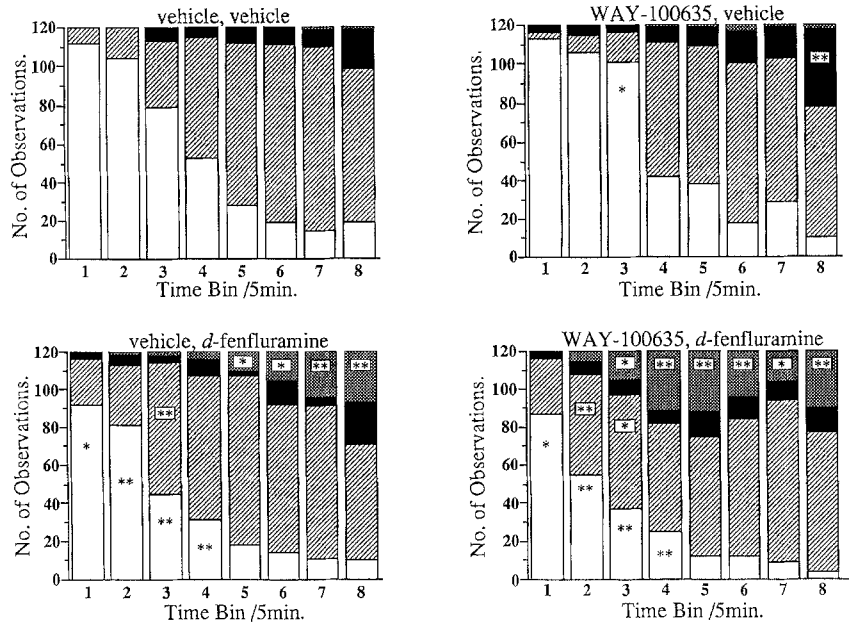


Fig. 2 The effects of *d*-fenfluramine (3.0 mg/kg) and WAY-100635 (1.00 mg/kg) pretreatment on the temporal organisation of consummatory and other behaviours. Twelve animals were individually observed every 2.5 s for a 40-min observational period. Hence, 120 behavioural observations were made in each 5-min time bin. One of four mutually exclusive behaviours was scored; feeding □, active ▨, grooming ■, resting ▩. * $P < 0.05$, ** $P < 0.01$, all comparisons to vehicle/vehicle condition

with the concomitant development of grooming behaviour, to a drug-induced advancement of satiety. Furthermore, *d*-fenfluramine tends to lead to hypoactivity in various test situations (Rowland and Carlton 1986). Grooming levels were greatest towards the end of the test period. WAY-100635 significantly enhanced grooming [$F(1, 11) = 6.83$, $P < 0.05$], an effect that was most prominent in the final time bin, and although subsequent administration of *d*-fenfluramine tended to restore grooming levels to control values, the antag × drug interaction was not significant [$F(1, 11) = 1.46$, n.s.]. The frequency of resting increased over the observation period [$F(7, 77) = 5.90$, $P < 0.001$]. *d*-Fenfluramine treatment (Fig. 2, bottom left panel), enhanced resting throughout the observation period and accordingly there was a main effect of drug [$F(1, 11) = 13.33$, $P < 0.005$]. In addition, there were significant effects of WAY-100635 with time [$F(7, 77) = 2.32$, $P < 0.05$] and a marginally significant three-way interaction between antag × time × drug [$F(7, 77) = 2.14$, $P < 0.05$]. This indicates that WAY-100635 tended to potentiate the enhancement of resting subsequent to *d*-fenfluramine treatment (see Fig. 2, bottom right panel). Such a pattern of results is consistent with the idea that *d*-fenfluramine treatment results in a temporal advance in the behavioural satiety sequence and pretreatment with WAY-100635 does not reverse this effect.

Experiment 2: the effects of metergoline on *d*-fenfluramine-induced changes in the behavioural satiety sequence

As in experiment 1, during the test period control animals exhibited the characteristic behavioural satiety sequence (Fig. 3, top left panel). *d*-Fenfluramine treatment significantly reduced mash consumption [$F(1, 10) = 59.01$, $P < 0.001$] and this was attenuated by pretreatment with metergoline [Fig. 1b: $F(1, 10) = 8.52$, $P < 0.05$].

Animals treated with *d*-fenfluramine exhibited a reduced incidence of feeding during each 5-min time bin. Consequently, there was a significant main effect of drug [$F(1, 10) = 58.17$, $P < 0.001$]. Pretreatment with metergoline restored the fenfluramine-induced decrement in feeding towards control values and led to a significant interaction between antagonist and drug [Fig. 3, bottom panels: $F(1, 10) = 6.02$, $P < 0.05$]. The interaction between antag × time × drug was not significant [$F(7, 70) = 1.31$, n.s.]. *d*-Fenfluramine had no significant effect on the total amount of active behaviours [$F(1, 10) = 0.150$, n.s.], though the interaction between drug × time was significant [$F(7, 70 = 6.74$, $P < 0.001$]. Metergoline pretreatment had no significant effect on either of these observations. The drug × time interaction is due both to the enhanced period of activity occuring in the initial bins and the reduced period of activity in later time bins as compared to control animals (Fig. 3, left panels). The earlier incidence of active behaviours in *d*-fenfluramine-treated animals is most likely due to the premature offset of feeding and likewise the reduced incidence of activity in later time bins is due to the advanced nature of resting behaviour. Again, this explanation is more convincing than the notion that the early offset of feeding may be a consequence of enhanced activity over these same initial periods as the incidence of grooming and resting behaviour are also temporally advanced (Fig. 3, bottom left panel). As in experiment 1, grooming levels were prevalent towards the end of the test. *d*-Fenfluramine treatment enhanced the level of grooming 20–30 min into the observation period

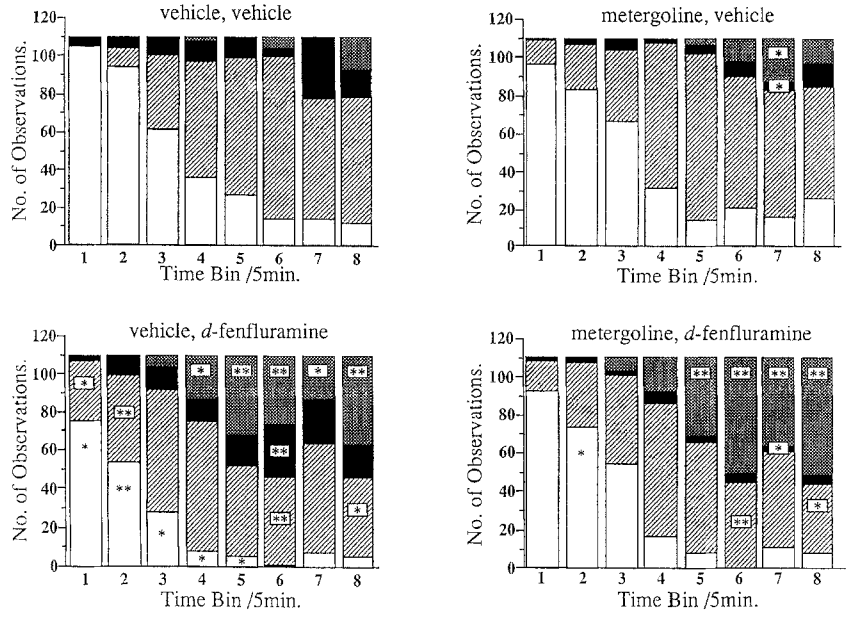


Fig. 3 The effects of *d*-fenfluramine (3.0 mg/kg) and metergoline (1.0 mg/kg) pretreatment on the temporal organisation of consummatory and other behaviours. Eleven animals were individually observed every 2.5 s for a 40-min observational period. Data from one animal which did not habitutate to the experimental protocol were excluded. Hence, 110 behavioural observations are presented in each 5-min time bin. One of four mutually exclusive behaviours was scored; feeding □, active ▨, grooming ■, resting ▩. * $P < 0.05$, ** $P < 0.01$, all comparisons to vehicle/vehicle condition

[drug × time $F(7, 70) = 2.15$, $P < 0.05$], whilst metergoline treatment significantly reduced the incidence of grooming, especially in later time bins [antag × time interaction $F(7, 70) = 4.58$, $P < 0.001$]. There was a marginally significant three-way interaction [$F(7, 70) = 2.44$, $P < 0.05$] indicating that *d*-fenfluramine treatment tended to potentiate the reduction in grooming induced by metergoline. The incidence of resting behaviour after both *d*-fenfluramine and metergoline treatment was significantly enhanced in later time bins [drug × time $F(7, 70) = 6.88$, $P < 0.001$; antag × time $F(7, 70) = 2.76$, $P < 0.05$]. Neither the antag × drug or antag × time × drug interactions were significant. *d*-Fenfluramine appears to enhance resting behaviour at the expense of feeding, whilst metergoline has a similar effect on resting at the expense of grooming behaviour (Fig. 3, bottom left and top right panels). As in experiment 1, the pattern of results obtained after *d*-fenfluramine treatment is conducive to the idea that *d*-fenfluramine treatment enhances satiety. Pretreatment with metergoline attenuated the *d*-fenfluramine-induced reduction in feeding behaviour, yet had little effect on the advanced onset of resting.

Experiment 3: attenuation of 8-OH-DPAT-induced hypophagia by WAY-100635

8-OH-DPAT significantly reduced wet mash intake in the 40-min test [$F(1, 49) = 72.14$, $P < 0.001$] and this effect was blocked by pretreatment with WAY-100635 (0.003–1.0 mg/kg) [Fig. 4: $F(6, 49) = 17.61$, $P < 0.001$]. At doses of 0.03–1.0 mg/kg, WAY-100635 fully attenuated the anorexia induced by 0.5 mg/kg 8-OH-DPAT.

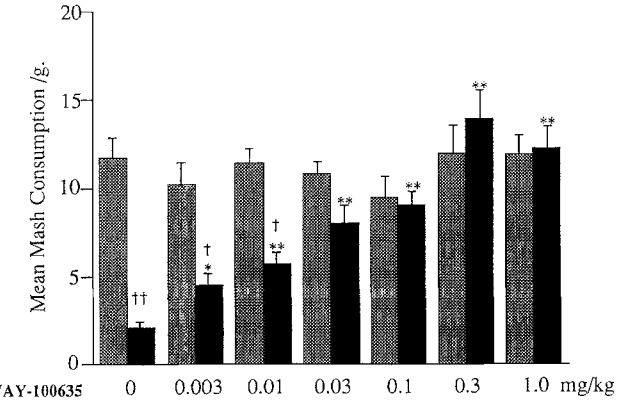


Fig. 4 The effect of WAY-100635 administration on wet mash in take over a 40-min period following injection of 0.5 mg/kg 8-OH-DPAT ■ or saline ▩ ($n = 8$). * $P < 0.05$, ** $P < 0.01$, as compared to vehicle/8-OH-DPAT condition. † $P < 0.05$, †† $P < 0.01$, comparisons between vehicle and 8-OH-DPAT treatments at each dose of WAY-100635. WAY-100635 was injected 30-min prior to drug treatment

Discussion

d-Fenfluramine treatment led to a temporal advance in the offset of feeding and onset of resting whilst preserving the qualitative pattern of behaviour characteristic of the behavioural satiety sequence (Fig. 2 and Fig. 3, bottom left panels). Such data indicate that *d*-fenfluramine acts so as to enhance satiety and is in concordance with previous reports (Blundell and McArthur 1978; Blundell and Latham 1980b; Halford and Blundell 1993). In addition, our findings are in accord with meal pattern studies which have demonstrated that *d*-fenfluramine treatment reduces meal size

while having no effect on meal frequency (Burton et al. 1981; Davies et al. 1983; Grignaschi et al. 1992); a conventional interpretation of such data is that satiation is enhanced by fenfluramine treatment.

Conversely, several studies (Montgomery and Willner 1988; Willner et al. 1990) have reported that fenfluramine treatment suppresses the onset of postprandial resting so arguing that the reduction in feeding is not compatible with an enhancement of satiety; though the same laboratory subsequently reported that the onset of postprandial resting was advanced on chronic fenfluramine treatment (McGuirk et al. 1992). While there is no obvious explanation for the apparent, cross-laboratory, discrepancy in the incidence of resting behaviour after fenfluramine treatment, each of these studies report data from food-deprived animals, whereas in the present studies animals were non-deprived.

Some authors (Samanin et al. 1989; Grignaschi and Samanin 1992) have eliminated 5-HT_{1A} receptor involvement in mediating the anorectic effect of *d*-fenfluramine on the basis that stimulation of presynaptic 5-HT_{1A} receptors has been associated with increased food intake (Dourish et al. 1985a; Gilbert and Dourish 1987). In experiment 3, the hypophagia elicited by a high dose of 8-OH-DPAT was fully blocked by pretreatment with WAY-100635 (Fig. 4). The experiment illustrates that a reduction of feeding can be brought about by a dose of 8-OH-DPAT that probably activates postsynaptic 5-HT_{1A} receptors. Furthermore, 8-OH-DPAT has been reported to decrease food intake in fasted rats at doses which do not elicit behavioural stereotypy (Ebenezer 1992) and recent evidence suggests that the hypophagia induced by CCK treatment is mediated by the 5-HT_{1A} receptor (Voigt et al. 1995). It is therefore feasible that the reduction in feeding observed after *d*-fenfluramine treatment could be mediated either wholly or in part by the 5-HT_{1A} receptor subtype.

The results from experiment 1 demonstrate that the 5-HT_{1A} antagonist WAY-100635 had no effect on the reduction in wet mash intake induced by *d*-fenfluramine. In addition, WAY-100635 had no effect on the temporally advanced behavioural sequence occurring subsequent to *d*-fenfluramine treatment (Fig. 2, bottom panels). These results indicate that the role of the 5-HT_{1A} receptor in mediating *d*-fenfluramine anorexia is negligible and are consistent with food intake data recently reported with racemic fenfluramine (Hartley et al. 1995).

Treatment with WAY-100635 increased grooming; most prominently in the final time bin. Jacobs (1991) has shown in the freely moving cat that approximately 30% of dorsal raphé neurones increase their firing during repetitive motor activities such as grooming with the tongue. The dorsal raphé nucleus is rich in 5-HT_{1A} binding sites (Verge et al. 1985). The application of WAY-100635 increases raphé cell firing in cats (Fornal et al. 1994) and may thereby interfere with the natural modulation of motor patterns involved in body licking. However, subsequent experiments performed in our laboratory have not replicated the enhancement of grooming after WAY-100635 administration observed in this experiment (unpublished results).

In combination, WAY-100635 and *d*-fenfluramine tended to potentiate the incidence of resting during the satiety sequence (Fig. 2, bottom right panel). In fact, there is a trend to suggest that WAY-100635 pretreatment potentiates the fenfluramine-induced advancement of the behavioural satiety sequence. One possible explanation of this finding is that *d*-fenfluramine treatment promotes the release of 5-HT, some of which will excite those 5-HT_{1A} receptors whose activation leads to increased feeding. WAY-100635 pretreatment will inhibit any such hyperphagic component after the administration of *d*-fenfluramine. Thus, the anorexia resulting from *d*-fenfluramine treatment may be greater in magnitude when given in conjunction with a 5-HT_{1A} antagonist.

As previously reported (Neill and Cooper 1989), metergoline treatment attenuated the reduction of wet mash intake induced by *d*-fenfluramine. Animals receiving this treatment also spent a greater period of time feeding than when treated with *d*-fenfluramine alone (Fig. 3, bottom right panel). Metergoline has been reported to increase food intake in satiated rats (Dourish et al. 1989) though there appears to be no evidence for such an effect under the experimental conditions used in these studies. Indeed metergoline-treated animals spent less time feeding in the first five time bins compared to controls (Fig. 3, top right panel). These findings are in agreement with Lee and Clifton (1992), who reported that an identical dose of metergoline slightly slowed feeding rate whilst having no significant effect on total intake.

Metergoline reduced the incidence of grooming both when given alone and in conjunction with *d*-fenfluramine (Fig. 3, right panels). The low levels of grooming may, in part, be a consequence of the enhanced levels of resting behaviour after metergoline treatment (Fig. 3, right panels). Metergoline increased the incidence of resting under conditions where feeding was less than controls. Thus, the effect on resting cannot be a consequence of increased feeding. The ability of metergoline to antagonise the reduced feeding but not the advanced onset of resting observed in *d*-fenfluramine treated animals might suggest that *d*-fenfluramine advances the behavioural satiety sequence by pharmacologically dissociable mechanisms. Such an interpretation would have important implications for the utility of the behavioural satiety sequence as an indicator of postprandial satiety. Thus, rather than potentiating endogenous satiety, *d*-fenfluramine may act so as to depress feeding and increase resting by distinct mechanisms. Alternatively, this apparent "dissociation"

may be due to properties of metergoline. In particular, metergoline has been reported to act as a partial agonist at the 5-HT_{1A} somatodendritic autoreceptor (Sharp et al. 1989). Such partial agonist properties may explain the reduction in grooming and the potentiation in resting observed after treatment in conjunction with *d*-fenfluramine. For example, the activity of serotonergic neurones projecting from the raphé has been found to be low during periods of inactivity and sleep (Jacobs and Fornal 1993). Indeed, the 5-HT_{1A} receptor agonist 8-OH-DPAT has been reported to decrease the incidence of grooming behaviour in rats (Montgomery et al. 1988).

The results of these studies provide unequivocal evidence that the 5-HT_{1A} receptor is not involved in mediating *d*-fenfluramine-induced anorexia. The use of ligands that are selective for 5-HT_1 and 5-HT_2 receptor subtypes other than the 5-HT_{1A} receptor may prove crucial in elucidating which receptor subtype(s) is/are of principal importance in mediating *d*-fenfluramine-induced anorexia. Specifically, studies utilising the 5-$HT_{2C/2B}$ antagonist SB 200646, which has 80-fold selectivity for this receptor site as opposed to all other neurotransmiter receptors (Kennett et al. 1994), and the 5-$HT_{1B/1D}$ antagonist GR 127935, which has little or no affinity for 5-HT_3 and 5-HT_4 receptors, though moderate affinity at 5-HT_{1A} and 5-HT_2 sites (Skingle et al. 1994), may prove particularly useful. Indeed, a preliminary report has demonstrated that *dl*-fenfluramine-induced anorexia is attenuated by pretreatment with SB 200646 but not GR 127935 (Hartley et al. 1995), suggesting that the role of the 5-HT_{1B} receptor in mediating this anorexia is only minor. This finding is in contrast to previous reports (Neill and Cooper 1989; Grignaschi and Samanin 1992). A more extensive study with such selective antagonists should resolve this issue. Furthermore, studies utilising recently described 5-HT_{1B} (Saudou et al. 1994) and 5-HT_{2C} (Tecott et al. 1995) knockout mice should prove useful in resolving which receptor subtype(s) is/are of principal importance in mediating *d*-fenfluramine induced anorexia and, in addition, elucidating serotonergic mechanisms of satiety.

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