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Investigation of the mechanisms underlying the hypophagic effects of the 5-HT and noradrenaline reuptake inhibitor, sibutramine, in the rat

[1]Helen C. Jackson, M. Clair Bearham, Lisa J. Hutchins, Sarah E. Mazurkiewicz, Andrew M. Needham & David J. Heal

Knoll Pharmaceuticals Research & Development, Pennyfoot Street, Nottingham, NG1 1GF

1 Sibutramine is a novel 5-hydroxytryptamine (5-HT) and noradrenaline reuptake inhibitor (serotonin-noradrenaline reuptake inhibitor, SNRI) which is currently being developed as a treatment for obesity. Sibutramine has been shown to decrease food intake in the rat. In this study we have used a variety of monoamine receptor antagonists to examine the pharmacological mechanisms underlying sibutramine-induced hypophagia.

2 Individually-housed male Sprague-Dawley rats were maintained on reversed phase lighting with free access to food and water. Drugs were administered at 09 h 00 min and food intake was monitored over the following 8 h dark period.

3 Sibutramine (10 mg kg^{-1}, p.o.) produced a significant decrease in food intake during the 8 h following drug administration. This hypophagic response was fully antagonized by the α_1-adrenoceptor antagonist, prazosin (0.3 and 1 mg kg^{-1}, i.p.), and partially antagonized by the β_1-adrenoceptor antagonist, metoprolol (3 and 10 mg kg^{-1}, i.p.) and the 5-HT receptor antagonists, metergoline (non-selective; 0.3 mg kg^{-1}, i.p.); ritanserin (5-$HT_{2A/2C}$; 0.1 and 0.5 mg kg^{-1}, i.p.) and SB200646 (5-$HT_{2B/2C}$; 20 and 40 mg kg^{-1}, p.o.).

4 By contrast, the α_2-adrenoceptor antagonist, RX821002 (0.3 and 1 mg kg^{-1}, i.p.) and the β_2-adrenoceptor antagonist, ICI 118,551 (3 and 10 mg kg^{-1}, i.p.) did not reduce the decrease in food intake induced by sibutramine.

5 These results demonstrate that β_1-adrenoceptors, 5-$HT_{2A/2C}$-receptors and particularly α_1-adrenoceptors, are involved in the effects of sibutramine on food intake and are consistent with the hypothesis that sibutramine-induced hypophagia is related to its ability to inhibit the reuptake of both noradrenaline and 5-HT, with the subsequent activation of a variety of noradrenaline and 5-HT receptor systems.

Keywords: Sibutramine; food intake; monoamine reuptake inhibition; serotonin-noradrenaline reuptake inhibitor (SNRI); noradrenaline; 5-hydroxytryptamine; α_1-adrenoceptors; β_1-adrenoceptors; 5-HT receptors

Introduction

Sibutramine HCl (BTS 54 524; *N*-{1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl}-*N,N*-dimethylamine hydrochloride monohydrate; Reductil; Meridia) is a novel 5-hydroxytryptamine (5-HT) and noradrenaline reuptake inhibitor *in vivo* (Buckett *et al*., 1988; Luscombe *et al*., 1989) and is, therefore, a member of a new class of drugs called the serotonin-noradrenaline reuptake inhibitors or SNRIs. Animal studies have shown that sibutramine decreases food intake in rats (Fantino & Souquet, 1995; Stricker-Krongrad *et al*., 1995) by enhancing the normal expression of satiety (Halford *et al*., 1995). It has also been demonstrated to produce dose-dependent, long lasting weight reduction in obese patients (Weintraub *et al*., 1991; Ryan *et al*., 1995) and is currently being developed to treat obesity.

The hypophagic effects of sibutramine would appear to be due to its ability to inhibit 5-HT and noradrenaline reuptake. This hypothesis is supported by our recent observations that food intake is decreased in rats by other 5-HT and noradrenaline reuptake inhibitors, such as venlafaxine and duloxetine, and by co-administration of doses of the 5-HT reuptake inhibitor, fluoxetine, and the noradrenaline reuptake inhibitor, nisoxetine, which were inactive when given alone (Jackson *et al*., 1996b).

Both 5-HT and noradrenaline are known to play important roles in the control of food intake (Rowland *et al*., 1996). Their actions are mediated by a variety of different receptor subtypes. In this study we have used a number of different monoamine receptor antagonists to explore the pharmacological mechanisms underlying the hypophagic effects of sibutramine in rats. These compounds include the α_1-adrenoceptor antagonist, prazosin; the α_2-adrenoceptor antagonist, RX821002 (Doxey *et al*., 1985); the β_1-adrenoceptor antagonist, metoprolol; the β_2-adrenoceptor antagonist, ICI 118,551 (Bilski *et al*., 1983) and the 5-HT receptor antagonists metergoline (non-selective), ritanserin (5-$HT_{2A/2C}$) and SB200646 (5-$HT_{2B/2C}$; Kennett *et al*., 1994).

Some of the results of this study have previously been published in abstract form (Jackson *et al*., 1996a).

Methods

Animals and environment

Experiments were performed on male Sprague-Dawley rats (350–500 g at the start of the experiment) which were obtained from Charles River (Margate). Animals were individually-housed in polypropylene cages with metal grid floors at a temperature of $21 \pm 1°C$ and 55% humidity. Polypropylene trays were placed below each cage to detect any food spillage. Animals were maintained on a reverse phase light-dark cycle. Lights were off from 09 h 00 min to 17 h 00 min during which time the laboratory was illuminated by a red lamp. Animals had free access to a standard powdered rat

[1] Author for correspondence.

diet (Compound Rat and Mouse Diet, Special Diet Services, Witham, Essex) and tap water at all times. The powdered diet was contained in glass feeding jars (10 cm diameter; 8 cm deep; Solmedia Laboratory Supplies, Romford) with aluminium lids. Each lid had a hole (3 cm diameter) cut in it to allow access to the food. Spillage of powdered diet from the feeding jars was negligible. Animals were accustomed to these conditions for at least two weeks before experimentation began.

Experimental procedures

On the test day, animals were randomly allocated to treatment groups containing 5–8 rats. Food intake was monitored during the dark phase since animals consume most of their food intake during the nocturnal period, and hence, the effects of drugs which suppress food intake can be readily detected. Feeding jars were weighed (to the nearest 0.1 g on a Sartorius L2200P top-pan balance) at the time of drug administration (09 h 00 min) and after 2, 4 and 8 h. The 8 h reading was taken immediately before the lights came on at 17 h 00 min. Animals were given sibutramine at a dose of 10 mg kg^{-1}, p.o. Antagonists were given by the i.p. route (with the exception of SB200646 which was given p.o.) at the same time as sibutramine. Four treatment groups were used in each experiment. The vehicle control group received the appropriate vehicle for the test drug and the antagonist. The drug control group received the test drug and the vehicle for the antagonist. The two other treatment groups received test drug plus one of two different doses of the antagonist. Doses of antagonists were based on effective doses obtained in previous behavioural experiments in our laboratory. Food intake of the four treatment groups was monitored concurrently, i.e. each experiment was completed in a single day. The effects of the monoamine receptor antagonists alone on food intake were investigated in separate experiments. Variations in body weight were accounted for by expressing the results as g kg^{-1} rat weight (treatment group means and s.e.mean). Rats in the weight-range used in this study would normally eat 15–20 g over the 8 h dark period, i.e. 30–50 g kg^{-1}. Animals were then divided into groups at random and re-used in the feeding studies after a wash-out period of at least 72 h.

Drugs

Drugs were: sibutramine hydrochloride (BTS 54 524; synthesized at Knoll Pharmaceuticals Research & Development, Nottingham), prazosin hydrochloride (Research Biochemicals International, St Albans), RX821002 hydrochloride (2-methoxy-idazoxan; Research Biochemicals International, St Albans), ICI 118,551 (1-[2,3-dihydro-(7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride; gift from Zeneca Pharmaceuticals, Macclesfield), metoprolol tartrate (Sigma, Poole), metergoline (gift from Farmitalia, Milan), ritanserin (Research Biochemicals International, St Albans) and SB200646 (*N*-(1-methyl-1H-indol-5-yl)-*N*′-(pyrid-3-yl) urea; synthesized at Knoll Pharmaceuticals Research & Development, Nottingham). Sibutramine was dissolved in deionized water. Prazosin, RX821002, metoprolol and ICI 118,551 were dissolved in deionized water or 0.9% saline. Metergoline and ritanserin were dissolved in deionized water minimally acidified with glacial acetic acid. SB200646 was suspended in deionized water plus a drop of diethylene glycol. All drug doses are expressed as the salt and drugs were administered in a dose volume of 1 ml kg^{-1}.

Statistical analysis

Statistical comparisons between the food intake of the different treatment groups were made by one-way analysis of variance followed by the Dunnett's multiple comparisons test (two-tailed).

Results

Effect of the α_1-adrenoceptor antagonist, prazosin, and the α_2-adrenoceptor antagonist, RX821002, on the decrease in food intake induced by sibutramine in the rat

The decrease in food intake induced by sibutramine (10 mg kg^{-1}, p.o.) was significantly inhibited by prazosin (0.3 and 1 mg kg^{-1}, i.p.) in a dose-dependent manner (Figure 1a). The highest dose (1 mg kg^{-1}) completely antagonized sibutramine-induced hypophagia 2, 4 and 8 h after drug administration. A 0.3 mg kg^{-1} dose of prazosin completely antagonized the decrease in food intake induced by sibutramine at 4 h and partially-antagonized sibutramine-induced hypophagia at 8 h. By contrast, the α_2-adrenoceptor antagonist, RX821002, did not antagonize the hypophagia induced by sibutramine (10 mg kg^{-1}, p.o.) in the 8 h following drug administration (Figure 1b). Neither prazosin (0.3 and 1 mg kg^{-1}, i.p.) nor RX821002 (0.3, 1 mg kg^{-1}, i.p.) had any effect on food intake when given alone (Table 1).

Effect of the β_1-adrenoceptor antagonist, metoprolol, and the β_2-adrenoceptor antagonist, ICI 118,551, on the decrease in food intake induced by sibutramine in the rat

The decrease in food intake produced by sibutramine (10 mg kg^{-1}, p.o.) was partially inhibited by metoprolol (3 and 10 mg kg^{-1}, i.p.) as shown in Figure 2a. Rats treated with sibutramine and metoprolol ate significantly more than those in the sibutramine group, but significantly less than those in the vehicle-treated control group throughout the 8 h dark period. On the other hand, the β_2-adrenoceptor antagonist, ICI 118,551, did not antagonize the hypophagia induced by sibutramine (10 mg kg^{-1}, p.o.) over the 8 h dark period (Figure 2b). Metoprolol (3 and 10 mg kg^{-1}, i.p.) and ICI 118,551 (3 and 10 mg kg^{-1}, i.p.) had no effect on food intake when given alone (Table 1).

Effect of the 5-HT receptor antagonists, metergoline, ritanserin and SB200646, on the decrease in food intake induced by sibutramine in the rat

The decrease in food intake produced by sibutramine (10 mg kg^{-1}, p.o.) was partially inhibited by a 0.3 mg kg^{-1}, i.p.

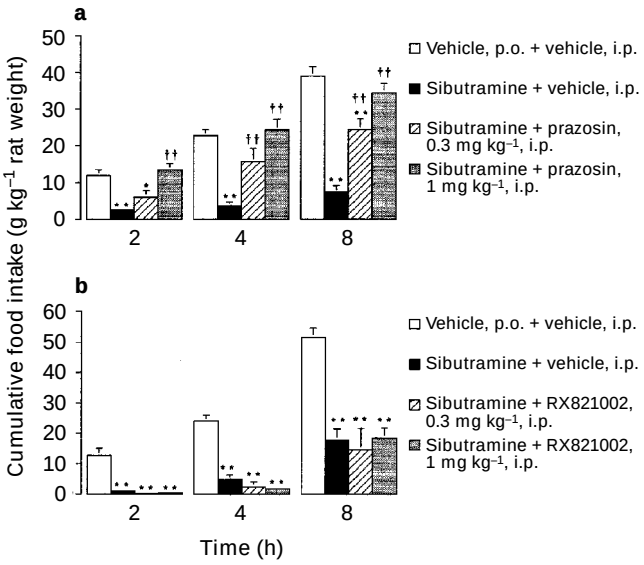

Figure 1 Effect of (a) the α_1-adrenoceptor antagonist, prazosin, and (b) the α_2-adrenoceptor antagonist, RX821002, on the decrease in food intake induced by sibutramine (10 mg kg^{-1}, p.o.) in the rat. Results are expressed as treatment group means for groups of 6–7 animals; vertical lines represent s.e.mean. Significant differences from the vehicle-treated control group are denoted by $*P<0.05$ and $**P<0.01$ and from the sibutramine-treated control group by $\dagger\dagger P<0.01$.

dose of the 5-HT receptor antagonist, metergoline (Figure 3a). This response was only observed at the 8 h reading. Cumulative 8 h food intakes of rats treated with sibutramine and metergoline 0.3 mg kg^{-1} were significantly different from those of animals treated with either vehicle or sibutramine. A higher dose of metergoline (1 mg kg^{-1}, i.p.) did not antagonize sibutramine-induced hypophagia during the 8 h dark period. The 5-HT$_{2A/2C}$ receptor antagonist, ritanserin (0.1 and 0.5 mg kg^{-1}, i.p.), partially inhibited the decrease in food intake induced by sibutramine (10 mg kg^{-1}, p.o.). Cumulative food intakes of animals given both sibutramine and ritanserin were significantly different from those of animals treated with either sibutramine or vehicle (Figure 3b). This effect was only apparent at the 8 h reading. The decrease in food intake produced by sibutramine (10 mg kg^{-1}, p.o.) was partially inhibited by the 5-HT$_{2B/2C}$ receptor antagonist, SB200646 (20 and 40 mg kg^{-1}, p.o.). These effects were only observed at the 8 h reading as shown in Figure 3c. Metergoline (0.3 and 1 mg kg^{-1}, i.p.); ritanserin (0.1 and 0.5 mg kg^{-1}, i.p.) and SB200646 (20 and 40 mg kg^{-1}, p.o.) had no effect on food intake when given alone (Table 2).

Table 1 Effect of α- and β-adrenoceptor antagonists on food intake in the rat

	Cumulative food intake (mg kg^{-1} rat weight) *Time after drug admininstration* (h)		
Treatment	*2*	*4*	*8*
Vehicle, i.p.	11.5±2.3	21.5±2.3	43.2±3.1
Prazosin 0.3 mg kg^{-1}, i.p.	16.8±1.7	28.2±1.9	49.6±2.0
Prazosin 1 mg kg^{-1}, i.p	12.6±1.7	23.1±2.6	44.2±2.5
Vehicle, i.p.	12.6±1.4	21.7±1.3	40.9±2.9
RX821002 0.3 mg kg^{-1}, i.p.	10.3±0.9	21.2±1.0	42.3±1.8
RX821002 1 mg kg^{-1}, i.p.	9.4±1.6	22.2±3.5	44.4±6.3
Vehicle, i.p.	10.8±2.5	19.5±4.8	35.0±5.9
Metoprolol 3 mg kg^{-1}, i.p.	11.7±1.5	17.1±1.0	36.7±0.9
Metoprolol 10 mg kg^{-1}, i.p.	10.8±1.4	16.8±1.0	38.8±1.6
Vehicle, i.p.	9.8±1.2	18.5±1.0	32.1±1.8
ICI 118,551 3 mg kg^{-1}, i.p.	10.4±0.6	20.0±1.4	33.1±1.4
ICI 118,551 10 mg kg^{-1}, i.p.	9.9±1.0	17.3±1.1	31.3±2.0

Values are means±s.e.mean for groups of 6–7 rats.

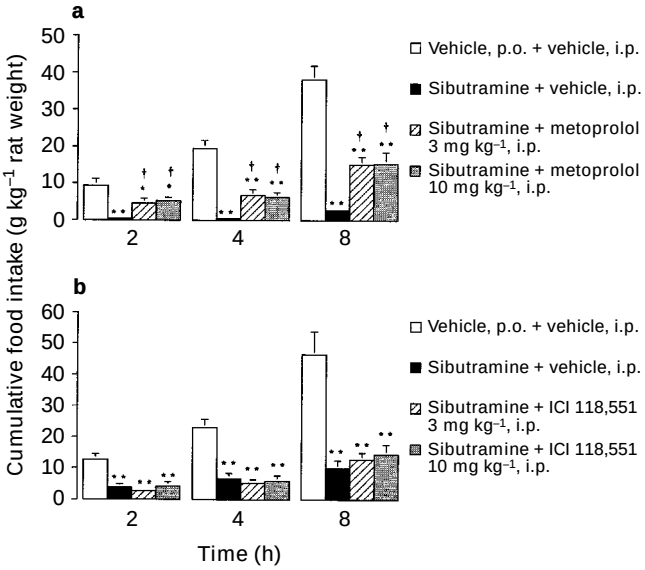

Figure 2 Effect of (a) the β_1-adrenoceptor antagonist, metoprolol, and (b) the β_2-adrenoceptor antagonist, ICI 118,551, on the decrease in food intake induced by sibutramine (10 mg kg^{-1}, p.o.) in the rat. Results are expressed as treatment group means for groups of 6–7 animals; vertical lines represent s.e.mean. Significant differences from the vehicle-treated control group are denoted by $*P<0.05$ and $**P<0.01$ and from the sibutramine-treated control group by $\dagger P<0.05$.

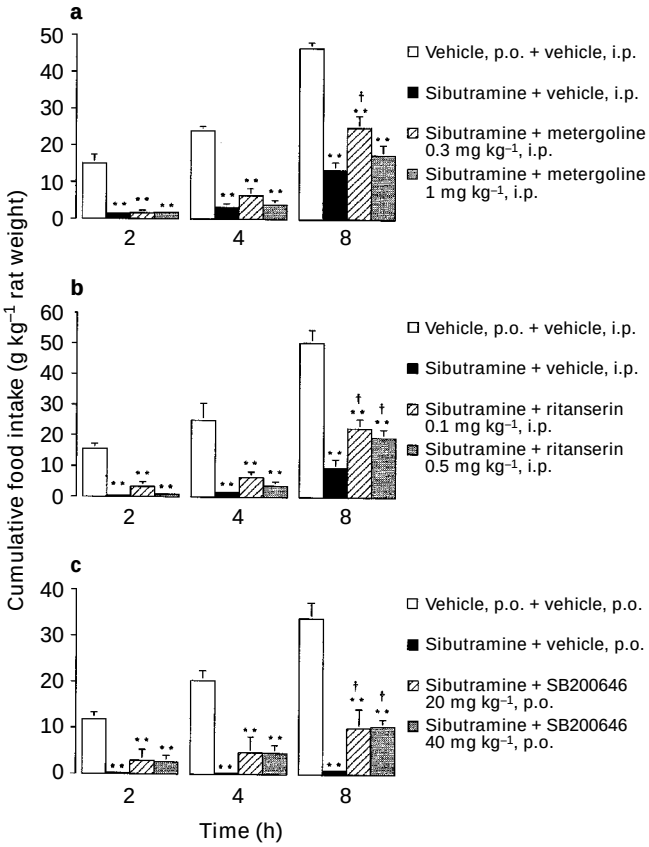

Figure 3 Effect of (a) the non-selective 5-HT receptor antagonist, metergoline; (b) the 5-HT$_{2A/2C}$ receptor antagonist, ritanserin, and (c) the 5-HT$_{2B/2C}$ receptor antagonist, SB200646, on the decrease in food intake induced by sibutramine (10 mg kg^{-1}, p.o.) in the rat. Results are expressed as treatment group means for groups of 5–8 animals; vertical lines represent s.e.mean. Significant differences from the vehicle-treated control group are denoted by $**P<0.01$ and from the sibutramine-treated control group by $\dagger P<0.05$.

Table 2 Effect of 5-HT receptor antagonists on food intake in the rat

	Cumulative food intake (mg kg^{-1} rat weight) *Time after drug admininstration* (h)		
Treatment	*2*	*4*	*8*
Vehicle, i.p.	13.3±1.6	24.2±1.1	41.3±0.5
Metergoline 0.3 mg kg^{-1}, i.p.	16.9±1.7	27.6±1.5	46.3±2.1
Metergoline 1 mg kg^{-1}, i.p.	13.0±1.8	23.7±1.7	43.7±2.0
Vehicle, i.p.	10.4±1.6	17.3±1.3	35.2±1.0
Ritanserin 0.1 mg kg^{-1}, i.p.	10.9±2.1	17.2±1.8	37.3±3.3
Ritanserin 0.5 mg kg^{-1}, i.p.	11.6±1.4	16.0±1.0	35.7±1.7
Vehicle, p.o.	17.5±1.8	26.0±2.0	42.7±2.6
SB200646 20 mg kg^{-1}, p.o.	18.7±3.1	27.9±3.9	47.6±4.7
SB200646 40 mg kg^{-1}, p.o.	14.7±2.5	21.6±2.6	36.9±3.8

Values are means±s.e.mean for groups of 6–8 rats.

Discussion

The hypophagic effects of the 5-HT and noradrenaline reuptake inhibitor, sibutramine, in rats were completely reversed by relatively low doses of the α_1-adrenoceptor antagonist, prazosin, which had no effect on food intake when given alone. Neither sibutramine, nor its primary and secondary amines (BTS 54 354 (Metabolite 1; *N*-{1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl}-*N*-methylamine hydrochloride) and BTS 54 505 (Metabolite 2; 1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride), which are thought to be predominantly responsible for the pharmacological actions of sibutramine *in vivo* (Luscombe *et al*., 1989), have affinity for α_1-adrenoceptors (S.C. Cheetham, personal communication). Hence, indirect activation of α_1-adrenoceptors subsequent to inhibition of noradrenaline reuptake would appear to play a prominent role in sibutramine-induced hypophagia. In support of this argument, the dose of sibutramine used in the present study is active in a number of behavioural models of noradrenaline reuptake inhibition (Luscombe *et al*., 1989). Moreover, dialysis studies have confirmed that sibutramine increases noradrenaline levels in rat brain (Stanford *et al*., 1997).

The hypothesis that α_1-adrenoceptors are involved in sibutramine-induced hypophagia is supported by studies showing that α_1-adrenoceptors are present in the paraventricular nucleus of the hypothalamus (Leibowitz *et al*., 1982), a brain area associated with control of food intake (Wellman *et al*., 1993). Furthermore, a number of different α_1-adrenoceptor agonists have been shown to suppress food intake in the rat (Davies & Wellman, 1992; Morien *et al*., 1993; Wellman *et al*., 1993).

α_2-Adrenoceptors have also been implicated in the control of food intake. They are present in the hypothalamus and α_2-adrenoceptor agonists such as clonidine have been shown to increase food intake in rats (Sanger, 1983; McCabe *et al*., 1984; Goldman *et al*., 1985). However, as shown in the current study, the hypophagic effects of sibutramine, were neither antagonized nor potentiated by the α_2-adrenoceptor antagonist, RX821002 (Doxey *et al*., 1985), confirming that these receptors are not involved in the effects of this drug on food consumption.

The suppressant effects of sibutramine on food intake were partially antagonized by doses of the selective β_1-adrenoceptor antagonist, metoprolol, which had no effect on food intake when given alone. Sibutramine and its major active metabolites have no affinity for β_1-adrenoceptors (S.C. Cheetham, personal communication), hence indirect activation of β_1-adrenoceptors would also appear to contribute to the effects of sibutramine on food intake.

A number of β-adrenoceptor agonists (including noradrenaline and isoprenaline) have been shown to decrease food intake in animals (Leibowitz & Rossakis, 1978). These observations are compatible with the involvement of β-adrenoceptors in sibutramine-induced hypophagia. The relative role of β_1-adrenoceptors in the control of food intake is not clear, as the effects of selective β_1-adrenoceptor agonists and antagonists on feeding behaviour have not been extensively investigated. On the other hand, activation of β_2-adrenoceptors in the perifornical hypothalamus has been shown to decrease food consumption in rats (Leibowitz & Rossakis, 1978; Bendotti *et al*., 1986). This mechanism does not appear to be responsible for the effects of sibutramine on food intake, since the hypophagia induced by this compound, was not attenuated by ICI 118,551, a selective β_2-adrenoceptor antagonist (Bilski *et al*., 1983) which readily crosses the blood-brain barrier (O'Donnell *et al*., 1994).

Interestingly, metroprolol antagonized sibutramine-induced hypophagia in the first few hours following drug administration, i.e. its time course of action was similar to that of prazosin. These results suggest that the decrease in food intake produced immediately after sibutramine treatment is largely mediated by adrenoceptors.

The hypophagic effects of sibutramine were partially antagonized by the 5-HT receptor antagonists, metergoline, ritanserin and SB200646, at doses that did not significantly alter food consumption over the 8 h dark period when given alone. The decrease in food intake produced by sibutramine was attenuated by a low, but not a high, dose of metergoline. These anomalous results may be explained by the limited selectivity of metergoline for different receptor subtypes. It has high affinity for 5-HT_{1A}, 5-$HT_{1B/1D}$, 5-HT_{2A} and 5-HT_{2C} receptors (Hoyer, 1988) and also exhibits moderate affinity for α_1-adrenoceptors and dopamine receptors (Leysen *et al*., 1981). Furthermore, although metergoline is an antagonist at 5-HT_{2A} and 5-HT_{2C} receptors (Hoyer *et al*., 1989; 1994), it has been shown to be an agonist at 5-HT_{1A} and 5-$HT_{1B/1D}$ receptors (Hoyer *et al*., 1994).

The inhibition of sibutramine-induced hypophagia by the low dose of metergoline and by ritanserin suggests that this reponse may be mediated, at least in part, by 5-$HT_{2A/2C}$ receptors. Ritanserin has much greater 5-HT_2/5-HT_1 receptor selectivity than metergoline (Leysen *et al*., 1985; Hoyer, 1988), although it still has moderate affinity for α_1-adrenoceptors and dopamine receptors (Leysen *et al*., 1985). Ritanserin acts as a 5-HT_{2A} and 5-HT_{2C} receptor antagonist *in vivo* (Leysen *et al*., 1985; Hoyer *et al*., 1989; 1994). However, there have been several studies showing that it does not inhibit the behavioural effects of 5-HT_{2C} receptor agonists (Kennett & Curzon, 1988; Knight & Fletcher, 1989), suggesting that it may be able to discriminate between 5-HT_{2A} and 5-HT_{2C} receptor function *in vivo*. It cannot be precluded, therefore, that ritanserin reduced sibutramine-induced hypophagia by antagonizing 5-HT_{2A}, as opposed to 5-HT_{2C} receptors. Studies with more selective 5-HT_{2A} receptor antagonists are required to confirm the role of 5-HT_{2A} receptors in sibutramine-induced hypophagia.

The attenuation of sibutramine-induced food intake by SB200646, which antagonizes 5-HT_{2B} and 5-HT_{2C} receptors (Kennett *et al*., 1994), demonstrates that 5-HT_{2C} receptors are also involved in the effects of sibutramine on food intake. It is unlikely that 5-HT_{2B} receptors play a role in sibutramine-induced hypophagia, as preliminary studies have shown that 5-HT_{2B} receptor agonists produce hyperphagia in rats (Ainsworth *et al*., 1996). The results with SB200646 are important, as unlike metergoline and ritanserin, SB200646 has negligible affinity for α_1-adrenoceptors (Kennett *et al*., 1994) and, therefore, its effects against sibutramine hypophagia can be completely dissociated from potential antagonist action at α_1-adrenoceptors.

In summary, the antagonist studies with metergoline, ritanserin and SB200646, implicate 5-HT_{2C}, and possibly 5-HT_{2A}, receptors in the hypophagic effects of sibutramine. Neither sibutramine nor its two amine metabolites exhibit affinity for 5-HT receptors (including the 5-HT_{2A} and 5-HT_{2C} receptor subtypes; S.C. Cheetham, personal communication). Therefore the effects of sibutramine on food intake would appear to be secondary, at least in part, to its ability to inhibit 5-HT reuptake *in vivo* (Buckett *et al*., 1988; Luscombe *et al*., 1989) with subsequent activation of 5-$HT_{2A/2C}$ receptors. This hypothesis is supported by the growing body of evidence that activation of the 5-HT system induces hypophagia in animals and man (Dourish, 1992; 1995) – a response which has been associated with 5-HT_{2A} and 5-HT_{2C} receptors following findings that directly-acting 5-HT_{2A} and 5-HT_{2C} receptor agonists produce a marked inhibition of feeding behaviour in rats (Clineschmidt *et al*., 1978; Schechter & Simansky, 1988; Kennett & Curzon, 1991).

Microdialysis studies have confirmed that sibutramine produces a small, but prolonged increase in extracellular levels of 5-HT in the hypothalamus of conscious rats (Gundlah *et al*., 1996; Prow *et al*., 1996). This response develops slowly following drug administration (Prow *et al*., 1996) – an observation which may explain why the three 5-HT receptor antagonists did not inhibit sibutramine-induced hypophagia until the second part of the 8 h dark period.

Further experiments are required to confirm the findings of this study which suggest that the noradrenaline system plays a prominent role in sibutramine-induced hypophagia in the first

few hours following drug administration whereas the 5-HT component does not fully emerge until a later stage. Sibutramine has been shown to enhance the satiety sequence of rats (measured 60–100 min following drug administration; Halford *et al*., 1995) and one approach would be to investigate the effects of monoamine receptor antagonists on this response.

The complete inhibition of sibutramine-induced hypophagia by prazosin is perhaps surprising in light of our results demonstrating that β_1-adrenoceptors and 5-$HT_{2A/2C}$ receptors also mediate at least part of the response. One possibility that warrants further investigation is that the α_1-adrenoceptors responsible for the hypophagic effects of sibutramine may be located downstream to the 5-HT and β_1-adrenoceptors.

In conclusion, the effects of sibutramine on food intake were fully antagonized by prazosin and partially antagonized by metoprolol, metergoline, ritanserin and SB200646, demonstrating that β_1-adrenoceptors; 5-$HT_{2A/2C}$ receptors and particularly α_1-adrenoceptors, are involved in the effects of sibutramine on food intake. These results are consistent with a preliminary study by Stricker-Krongrad *et al*. (1996) showing that the hypophagic effects of sibutramine were antagonized by metoprolol and ritanserin, but not by ICI 118,551, and support the concept that sibutramine-induced hypophagia is due to its ability to inhibit the reuptake of both noradrenaline and 5-HT, with the subsequent activation of a variety of noradrenaline and 5-HT receptor systems.

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