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Citalopram-induced hypophagia is enhanced by blockade of 5-HT$_{1A}$ receptors: role of 5-HT$_{2C}$ receptors

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1 The selective 5-hydroxytryptamine reuptake inhibitor citalopram (10 and 20 mg kg^{-1}, i.p.) significantly reduced food intake in male rats (CD-COBS) habituated to eat their daily food during a 4-h period.

2 The 5-HT$_{1A}$ receptor antagonist WAY100635 (0.3 mg kg^{-1}) administered systemically did not modify feeding but significantly potentiated the reduction in food intake caused by 10 mg kg^{-1} i.p. citalopram. The dose of 5 mg kg^{-1} i.p. citalopram was not active in animals pretreated with vehicle but significantly reduced feeding in animals pretreated with WAY100635.

3 WAY100635 (0.1 μg 0.5 μl^{-1}) injected into the dorsal raphe significantly potentiated the hypophagic effect of 10 mg kg^{-1} citalopram.

3 WAY100635 (1.0 μg 0.5 μl^{-1}) injected into the median raphe did not modify feeding or the hypophagic effect of 10 mg kg^{-1} citalopram.

5 The 5-HT$_{2B/2C}$ receptor antagonist SB206553 (10 mg kg^{-1}, p.o.) slightly reduced feeding by itself but partially antagonized the effect of WAY100635 administered systemically (0.3 mg kg^{-1}, s.c.) or into the dorsal raphe (0.1 μg 0.5 μl^{-1}) in combination with 10 mg kg^{-1} i.p. citalopram. The hypophagic effect of 10 mg kg^{-1} i.p. citalopram alone was not significantly modified by SB206553.

6 Brain concentrations of citalopram and its metabolite desmethylcitalopram in rats pretreated with SB206553, WAY100635 and their combination were comparable to those of vehicle-pretreated rats, 90 min after citalopram injection.

7 The hypophagic effect of citalopram was potentiated by blocking 5-HT$_{1A}$ receptors. Only the effect of the WAY100635/citalopram combination seemed to be partially mediated by central 5-HT$_{2C}$ receptors.

Keywords: Citalopram; SSRIs; food intake; drugs' brain concentrations; 5-hydroxytryptamine; 5-HT$_{1A}$ receptors; 5-HT$_{2C}$ receptors; WAY100635; SB206553

Introduction

Selective 5-hydroxytryptamine (5-HT) reuptake inhibitors (SSRIs) raise the extracellular concentrations of 5-HT in several brain regions (Fuller, 1994) including the raphe area where most 5-HT cell-bodies innervating the forebrain are found (Invernizzi *et al*., 1992; Bel & Artigas, 1992). Somatodendritic 5-HT$_{1A}$ receptors in the raphe nuclei appear to play an important role in controlling the ability of SSRIs to increase extracellular 5-HT. Blockade of these receptors by non-selective (Invernizzi *et al*., 1991, 1992; Hjorth, 1993; Bel *et al*., 1994) and selective 5-HT$_{1A}$ receptor antagonists (Gartside *et al*., 1995; Hjorth *et al*., 1996; Invernizzi *et al*., 1996; 1997a) facilitates the effect of SSRIs on extracellular 5-HT in various terminal regions of the rat brain with the exception of the dorsal hippocampus where rise of extracellular 5-HT is not affected or is only slightly enhanced by WAY100635, a selective 5-HT$_{1A}$ receptor antagonist (Invernizzi *et al*., 1997a; Romero & Artigas, 1997).

Only a few studies have provided functional evidence that the action of SSRIs is potentiated by 5-HT$_{1A}$ receptor antagonists (Sànchez, 1997; Hashimoto *et al*., 1997; Browning *et al*., 1997). Hypophagia is a commonly studied effect of SSRIs and fluoxetine and sertraline have been proposed as anorectic agents (Yen *et al*., 1987; Lucki *et al*., 1988). Thus, the question whether 5-HT$_{1A}$ receptor blockade enhances the effect of SSRIs on food intake, together with their antidepressant activity, is of particular interest. Our laboratory has found that the reduction in food intake caused by the SSRI citalopram was potentiated by WAY100635 (Invernizzi *et al*., 1997b). Similar results were reported by Trillat *et al*. (1997) using fluoxetine, while in another study (Ciccocioppo *et al*., 1997) the 5-HT$_{1A}$ receptor antagonist WAY100135 did not modify the hypophagic effect of fluoxetine.

In the present study we first examined the hypophagic effect of citalopram, a potent and selective SSRI with no apparent affinity for 5-HT receptors (Hyttel, 1977), using it alone or in combination with WAY100635 injected subcutaneously or into the raphe nuclei dorsalis (DR) and medianus (MR), with the aim of confirming that blockade of presynaptic 5-HT$_{1A}$ receptors facilitates the effect of citalopram on food intake.

In the second part of the study we explored the role of 5-HT$_{2C}$ receptors in the hypophagic effect of citalopram, alone or in combination with WAY100635, by using a selective antagonist at 5-HT$_{2B/2C}$ receptors, SB206553 (Forbes *et al*., 1995) at a dose active in reducing the hypolocomotor (Kennett *et al*., 1996) and the hypophagic (this study) response to m-chlorophenylpiperazine (mCPP), a 5-HT$_{2C}$ receptor agonist (Hoyer, 1988).

Methods

Animals

Male Sprague-Dawley rats (CD-COBS, Charles River, Italy) weighing 220–250 g were housed in groups of two in a room at 21 ± 1°C and 60% relative humidity, with a 12-h light/12-h

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dark cycle (light off at 19.00 h) and water *ad libitum*. Procedures involving animals and their care are conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., suppl. 40, 18 Febbraio 1992, Circolare No. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

Cannulae implantation

Rats were anesthetized with 3.0 ml kg^{-1} equithesin injected intraperitoneally (i.p.) (1.2 g of sodium pentobarbital; 5.3 g of chloral hydrate; 2.7 g of $MgSO_4$; 49.5 ml of propylene glycol; 12.5 ml of ethanol and 58 ml of distilled water) and placed on a stereotaxic apparatus (mod. 900, David Kopf, Tujunga, CA, U.S.A.). The skin was incised and the skull was cleaned. Guide cannulae made of 23-gauge stainless-steel tubing were implanted 2 mm above the site to be injected. The guide tubes were secured with acrylic dental cement anchored to three stainless-steel screws fixed to the skull. To prevent clogging, 30-gauge stainless-steel stylets were placed in the guide cannulae until the animals were given intracerebral injections. On the day of the test, the stylets were withdrawn and replaced by injection units (30-gauge stainless-steel tubing) terminating 2 mm below the tip of the guides. The drugs were infused over a period of 1 min, with an additional 30 s allowed for diffusion of the solution away from the needle tip.

Stereotaxic coordinates from the interaural line for the DR and MR were as follows: AP = +1.2, V = +3.2, L = 0 mm and AP = +1.2, V = +1.5, L = 0 mm (Paxinos & Watson, 1982). Cannulae were implanted at a 20° angle to the sagittal plane to avoid damage to the sinus.

Food intake

The animals were trained to eat their daily food (Altromin food pellets for rats) from 14.00 h to 18.00 h for 10 days. During the habituation period the animals were handled gently, weighed and injected i.p. with saline at 13.30 h, 30 min before food was made available. On the day of the experiment the rats were injected with the various drugs and at 14.00 h they were placed in individual Plexiglass cages containing a weighed amount of food in a Perspex Petri dish, with a paper towel below the cage floor to collect any spillage. Food intake in 30 min was measured by weighing all portions of food before and after the feeding test, and expressed as g 100 g^{-1} of body weight.

Experimental procedure

Experiment 1: effect of citalopram on food intake Citalopram was injected i.p., at doses between 5 and 20 mg kg^{-1}, 30 min before the test. All the animals were tested on the same day.

Experiment 2: effect of WAY100635 injected systemically on the reduction in food intake caused by citalopram The effect of WAY100635 injected systemically on citalopram-induced hypophagia was evaluated in conditions in which the 5-HT$_{1A}$ receptor antagonist enhanced the effect of citalopram on extracellular 5-HT in terminal regions (Invernizzi *et al*., 1997a). Doses of 5 and 10 mg kg^{-1} i.p. citalopram were given, together with 0.3 mg kg^{-1} s.c. WAY100635, 30 min before the test. The experiments were performed on two consecutive days with control- and drug-treated animals tested concurrently.

Experiment 3: effect of WAY100635 injected into the dorsal or median raphe on the reduction in food intake caused by citalopram How 5-HT$_{1A}$ receptors located in the raphe nuclei affect the hypophagic effect of citalopram was studied by injecting WAY100635 directly into the dorsal or median raphe. Thus, 10 mg kg^{-1} i.p. citalopram was given together with 0.1 and 1.0 μg 0.5 μl^{-1} WAY100635 into the dorsal raphe or 1.0 μg 0.5 μl^{-1} WAY100635 into the median raphe, immediately before the test.

The experiments were performed on two consecutive days with control- and drug-treated animals tested cuncurrently.

Experiment 4: effect of SB206553 on the reduction in food intake caused by WAY100635 plus citalopram How 5-HT$_{2B/2C}$ receptors affect the hypophagia caused by the combined treatment of citalopram (10 mg kg^{-1}) and WAY100635, administered systemically (0.3 mg kg^{-1}) or into the DR (0.1 μg 0.5 μl^{-1}), was studied in animals pretreated with SB206553 (10 mg kg^{-1}). This dose was selected on the basis of preliminary experiments showing that it reduced the hypophagic effect of 5 mg kg^{-1} mCPP, and that 20 mg kg^{-1} had a similar effect. The experiments were performed on four consecutive days with control- and drug-treated animals tested concurrently.

Drug measurements

Ninety minutes after citalopram injection animals were killed by decapitation under deep anaesthesia. Brains were rapidly removed, blotted with paper to remove excess surface blood and stored at −20°C until analysis.

Brain concentrations of citalopram and its metabolites desmethylcitalopram (DCIT) and didesmethylcitalopram (DDCIT) were determined by high-performance liquid chromatography with U.V. detection (235 nm). Briefly, citalopram, its metabolites and the internal standard were extracted from brain homogenate (distilled water, 1 g 10 ml^{-1}) with diethyl ether at pH 10 as described by Oyehaug *et al*. (1984). After centrifugation the ether layers were evaporated under a stream of nitrogen, redissolved in the mobile phase (see below) and injected into the chromatographic column (μBondapack C18, 30 cm × 3.9 mm i.d., 10 μm, held at room temperature).

The mobile phase was 0.025 M KH_2PO_4-CH_3CN-trietylamine (63.9 : 36 : 0.1 v v^{-1}, buffered to pH 6.7 with H_3PO_4, delivered isocratically at a flow rate of 1 ml min^{-1}. Approximate retention times were 13 min for DDCIT, 16 min for DCIT, 21 min for citalopram and 29 min for the internal standard.

The limit of detection was 0.1 μg g^{-1} for all compounds, using approximately 200 mg of brain tissue (2 ml of homogenate). At these concentrations the coefficient of variation (C.V.) was 10–11% for all compounds, and all higher concentrations gave C.V. less than 5%.

Drugs

Citalopram ([1-(3-dimethylamino)propyl]-1-(P-fluoro-phenyl)-5-phthalancarbonitrile HBr) (Lundbeck, Copenhagen-Valby, DK) and mCPP (1-(3-chlorophenyl)piperazine HCl) (Sigma-Aldrich, Steinheim, Germany) were dissolved in saline (2 ml kg^{-1}). WAY100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide 3HCl) (synthesized by Pharmacia-Upjohn, Milan, Italy) was dissolved in distilled water (2 ml kg^{-1}). SB206553 (5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo [2,3-f] indole)

(SmithKline Beecham, Harlow, U.K.) was suspended in carboymethylcellulose 0.5% (2 ml kg^{-1}).

Histology

The cannula placings were checked histologically after each experiment. Only data from rats in which the cannulae were exactly located were included in the results.

Statistical analysis

The reduction in food intake caused by citalopram was analysed by one-way analysis of variance followed by Dunnett's test. The effect of pretreatment with 5-HT receptor antagonists on the hypophagic effect of citalopram was analysed by factorial ANOVA for balanced or unbalanced designs (GLM procedure; SAS, SAS Institute, NC). Whenever the three-way interaction was significant, the effect of the 5-HT$_{2B/2C}$ receptor antagonist SB206553 on citalopram-induced hypophagia in rats pretreated with vehicle or WAY100635 was analyzed separately by two-way ANOVA. *Post hoc* comparisons were made by Tukey-Kramer's test.

Results

As shown in Figure 1, 10 and 20 mg kg^{-1} i.p. citalopram significantly reduced the amount of food eaten in 30 min by food-deprived rats, whereas 5 mg kg^{-1} had no such effect.

WAY100635 (0.3 mg kg^{-1} s.c.) had no effect by itself (Figure 2) but markedly potentiated the reduction in food intake caused by 10 mg kg^{-1} i.p. citalopram; 5 mg kg^{-1} citalopram, which had no effect by itself, significantly reduced food intake in rats pretreated with WAY100635 (Fint(2,34) = 6.9; $P<0.01$).

Figure 3 shows the effect of WAY100635 injected into the raphe nuclei on the hypophagia caused by citalopram. The 5-HT$_{1A}$ receptor antagonist injected into the DR (Figure 3a) at the doses of 0.1 μg 0.5 μl^{-1} significantly potentiated the hypophagia caused by 10 mg kg^{-1} i.p. citalopram (Fint(1,24) = 27.3; $P<0.01$). A higher dose of WAY100635 (1.0 μg 0.5 μl^{-1}) did not increase further the effect of citalopram and slightly but significantly reduced feeding by itself (data not shown).

WAY100635 injected into the MR (1.0 μg 0.5 μl^{-1}) had no effect by itself and did not influence the reduction in food intake caused by citalopram (Fint(1,27) = 0.46; $P>0.05$) (Figure 3b).

Figure 4 shows the effect of pretreatment with SB206553 (10 mg kg^{-1}, p.o.) on the reduction in food intake caused by citalopram (10 mg kg^{-1} i.p.) together with WAY100635. Three-way ANOVA indicated that the effect of citalopram plus WAY100635, injected systemically (0.3 mg kg^{-1} s.c.) (Figure 4a) or into the DR (0.1 μg 0.5 μl^{-1}) (Figure 4b), was significantly different in rats given SB206553 or vehicle (Fint(1,72) = 4.9; $P<0.05$ and Fint(1,51) = 5.9; $P<0.05$ respectively). Further analysis showed that 10 mg kg^{-1} p.o. SB206553 tended to reduce feeding by itself and in one experiment this reduction was statistically significant ($P<0.05$ Tukey-Kramer's test; Figure 4b). The dose of 10 mg kg^{-1} p.o. SB206553 did not antagonize the reduction in food intake caused by 10 mg kg^{-1} i.p. citalopram alone (Fint(1,39) = 0.32 and Fint(1,25) = 0.00 $P>0.05$ respectively for systemic and intra-DR injection) whereas it partially antagonized the potentiation of the hypophagic effect of 10 mg kg^{-1} i.p. citalopram caused by WAY100635, either systemically or into the DR (Fint(1,33) = 12.8; $P<0.01$ and Fint(1,28) = 5.9; $P<0.05$ respectively).

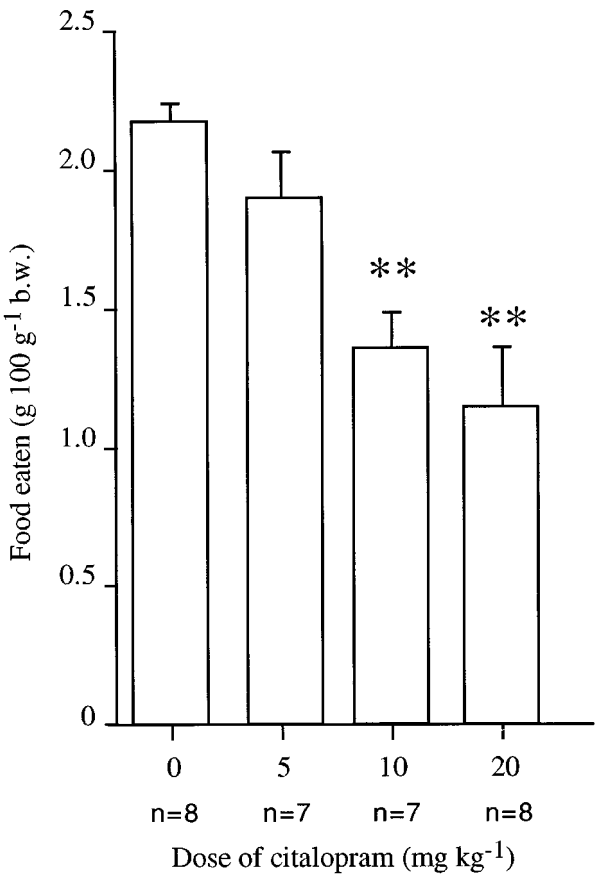

Figure 1 Effect of citalopram on food intake by food-deprived rats. Mean ± s.e.m. of at least four animals per group. Citalopram (5–20 mg kg^{-1} i.p.) was given 30 min before testing. ** = $P<0.01$ vs saline-treated group. Dunnett's test.

The animals given citalopram alone or in combination with the other drugs did not show any overt behavioural effects that could have interfered with eating.

Mean brain concentrations of citalopram and DCIT in rats treated with WAY100635 alone or with SB206553 were comparable to those in saline-pretreated animals. Citalopram concentrations (μg g^{-1}) were: vehicle + citalopram = 1.2 ± 0.5; WAY100635 + citalopram = 1.3 ± 0.5; WAY100635 + SB206553 + citalopram = 1.2 ± 0.5; DCIT concentrations (μg g^{-1}) were: vehicle + citalopram = 0.2 ± 0.1; WAY100635 + citalopram = 0.2 ± 0.0; WAY100635 + SB206553 + citalopram = 0.2 ± 0.1.

Brain concentrations of DDCIT were below the limit of sensitivity of the analytical procedure (0.1 μg g^{-1}, using approximately 200 mg of brain tissue).

Discussion

Citalopram dose-dependently reduced food intake but even the highest dose (20 mg kg^{-1}) reduced eating only by 50%. The reasons for this are not completely clear. We found that 20 mg kg^{-1} citalopram did not raise extracellular 5-HT any more than 10 mg kg^{-1} in terminal regions such as striatum and dorsal hippocampus (unpublished results). The fact that this higher dose had no further effect on the extracellular concentrations of 5-HT in forebrain regions involved in eating

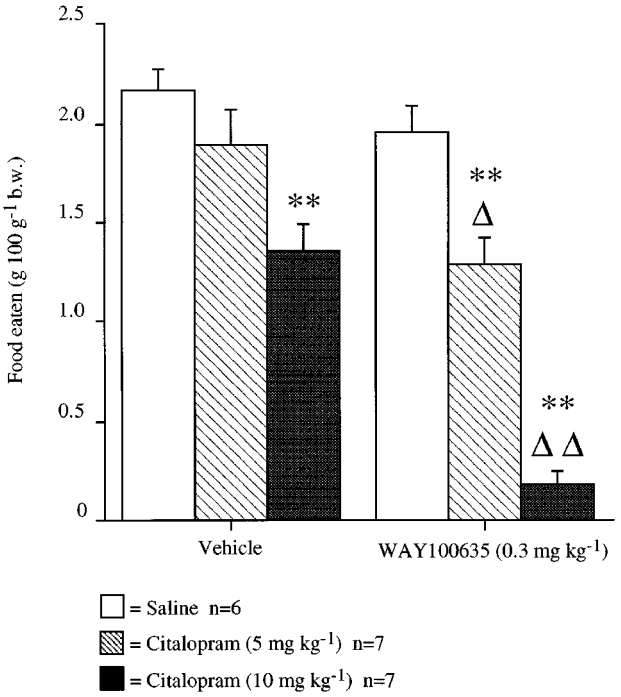

Figure 2 Effect of pretreatment with WAY100635 injected systemically on the reduction in food intake caused by citalopram in food-deprived rats. Mean ± s.e.m. of at least six animals per group. WAY100635 (0.3 mg kg^{-1} s.c.) and citalopram (5 and 10 mg kg^{-1} i.p.) were given 30 min before testing. ** = $P<0.01$ vs respective saline. Tukey-Kramer's test. Δ = $P<0.05$ ΔΔ = $P<0.01$ vs vehicle-treated group. Tukey-Kramer's test. Fint(2,34) = 6.9 $P<0.01$.

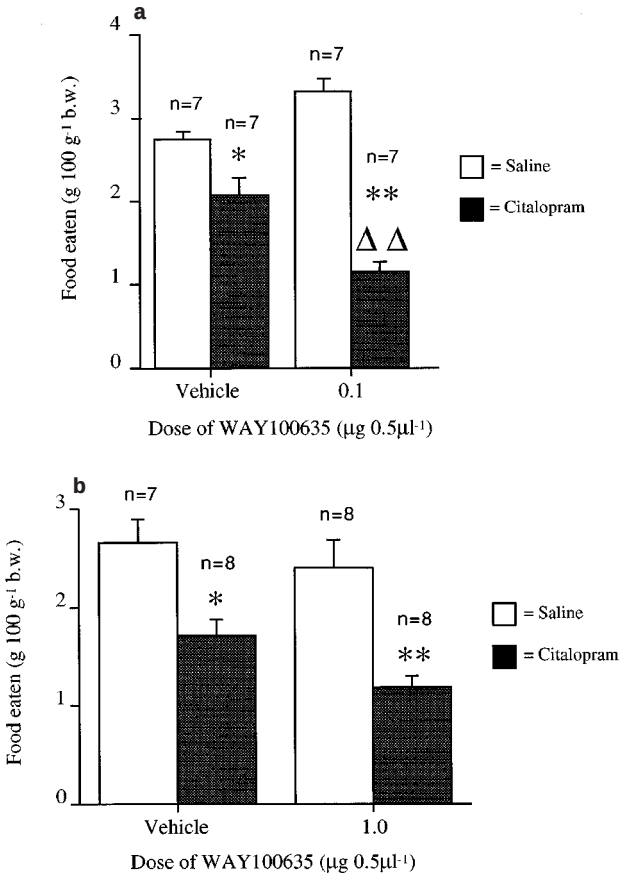

Figure 3 (a) Effect of WAY100635 injected into the DR on the reduction in food intake caused by citalopram. Mean ± s.e.m. of at least six animals per group. Citalopram (10 mg kg^{-1} i.p.) was given 30 min before testing. WAY100635 (0.1 μg 0.5 μl^{-1}) was injected into the DR immediately before testing. * = $P<0.05$; ** = $P<0.01$ vs respective saline. Tukey-Kramer's test. ΔΔ = $P<0.01$ vs vehicle + citalopram. Tukey-Kramer's test. Fint(1,24) = 27.3; $P<0.01$. (b) Effect of WAY100635 injected into the MR on the reduction in food intake caused by citalopram. Mean ± s.e.m. of at least six animals per group. Citalopram (10 mg kg^{-1} i.p.) was given 30 min before testing. WAY100635 (1.0 μg 0.5 μl^{-1}) was injected into the MR immediately before testing. * = $P<0.05$; ** = $P<0.01$ vs respective saline. Tukey-Kramer's test. Fint(1,27) = 0.46; $P>0.05$.

control may explain why it did not reduce rats' food intake more. Compatible with this is the finding that WAY100635, at a dose (0.3 mg kg^{-1}) increasing the effect of citalopram on extracellular 5-HT (Invernizzi *et al*., 1997a), significantly enhanced the effect of 10 mg kg^{-1} citalopram, causing about 90% reduction of food intake.

This effect could not be attributed to a kinetic interference since the brain levels of citalopram and its metabolite desmethylcitalopram in WAY100635-treated animals were no different from controls. These results clearly suggest that blockade of 5-HT$_{1A}$ receptors facilitates the hypophagic effect of a SSRI. A similar conclusion was reached in one preliminary study in which WAY100635 0.5 mg kg^{-1} given intravenously 1 h after i.p. fluoxetine potentiated the effect of the SSRI on extracellular 5-HT in ventral hippocampus and on food intake (Trillat *et al*., 1997).

In apparent contrast with these findings Ciccocioppo *et al*. (1997) reported that the 5-HT$_{1A}$ receptor antagonist WAY100135 did not modify fluoxetine's effect on food intake. Several studies have shown that WAY100135 reduces 5-HT release and the firing activity of 5-HT cells (Assié & Koek, 1996; Fornal *et al*., 1994; Escandon *et al*., 1994; Lanfumey *et al*., 1993) and the effect on 5-HT release was blocked by WAY100635 (Assié & Koek, 1996). The residual 5-HT$_{1A}$ receptor agonist activity of WAY100135 (Assié & Koek, 1996) does not guarantee an effective blockade of 5-HT-induced inhibition of 5-HT cells as shown by the finding that WAY100135 did not prevent the reduction of striatal 5-HT concentrations caused by citalopram injected into the DR (Hjorth *et al*., 1996). Thus WAY100135 may not have enhanced the effect of fluoxetine on forebrain extracellular 5-HT. Other confounding factors in the Ciccocioppo study are the use of alcohol-preferring rats and the fact that 5 mg kg^{-1} fluoxetine was very effective in reducing food intake in these animals, making it difficult to detect any potentiating effect by WAY100135.

Although 5-HT$_{1A}$ receptors are also found postsynaptically in various forebrain areas, particularly the cerebral cortex and hippocampus (Pazos *et al*., 1988), they are unlikely to have contributed to the potentiating effect of WAY100635. Besides the fact that blockade of postsynaptic 5-HT$_{1A}$ receptors does not modify the effect of citalopram on extracellular concentrations of 5-HT (Hjorth *et al*., 1996) it is well known that presynaptic 5-HT$_{1A}$ receptors are involved in the control of food intake (Bendotti & Samanin, 1986; Dourish *et al*., 1986). We have found that 0.1 μg WAY100653 injected directly into the DR significantly potentiated citalopram's effect on food intake. Blockade of 5-HT$_{1A}$ receptors in the DR may potentiate the hypophagic effect of citalopram, presumably by facilitating its effect on extracellular 5-HT in regions involved in feeding control. Although 5-HT$_{1A}$ receptors in the MR is also involved in the control of food intake (Bendotti &

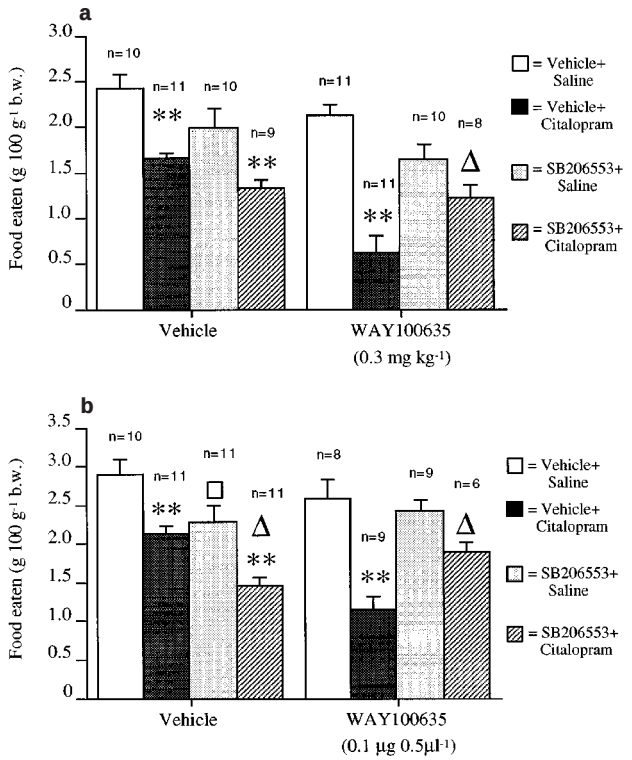

Figure 4 (a) Effect of pretreatment with SB206553 on the reduction in food intake caused by citalopram plus WAY100635 injected systemically. Mean ± s.e.m. of at least eight animals per group. SB206553 (10 mg kg^{-1} p.o.) was given 60 min before the test. WAY100635 (0.3 mg kg^{-1} s.c.) and citalopram (10 mg kg^{-1} i.p.) were given 30 min before testing. ** = $P<0.01$ vs respective saline. Tukey-Kramer's test. $\Delta = P<0.05$ vs vehicle + citalopram. Tukey-Kramer's test. Vehicle-treated rats: Fint(1,39) = 0.3 $P>0.05$ (Two-way ANOVA). WAY100635-treated rats: Fint(1,33) = 12.8 $P<0.01$ (two-way ANOVA). (b) Effect of pretreatment with SB206553 on the reduction in food intake caused by citalopram plus WAY100635 injected into the DR. Mean ± s.e.m. of at least six animals per group. SB206553 (10 mg kg^{-1} p.o.) was given 60 min before the test, citalopram (10 mg kg^{-1} i.p.) 30 min before testing. WAY100635 (0.1 μg 0.5 μl^{-1}) was injected into the DR immediately before the test. ** = $P<0.01$ vs respective saline. Tukey-Kramer's test. $\Delta = P<0.05$ vs vehicle + citalopram. $\square = P<0.05$ vs vehicle + saline. Tukey-Kramer's test. Vehicle-treated rats: Fint(1,25) = 0.0 $P>0.05$ (two-way ANOVA). WAY100635-treated rats: Fint(1,28) = 5.9 $P<0.05$ (two-way ANOVA).

Samanin, 1986), the highest dose of WAY100635 (1 μg) injected into this nucleus did not modify feeding by itself or citalopram's effect on food intake. This is in line with recent evidence that blockade of 5-HT$_{1A}$ receptors in the MR is much less effective than blockade of DR 5-HT$_{1A}$ receptors in enhancing the action of citalopram on extracellular 5-HT in their respective terminal regions (Invernizzi *et al*., 1997a; Romero & Artigas, 1997).

In the second part of this study we examined whether a selective 5-HT$_{2B/2C}$ receptor antagonist, SB206553, modified the effect on food intake of citalopram alone or in combination with WAY100635. SB206553 did not change the hypophagia caused by 10 mg kg^{-1} citalopram but partially antagonized the effect of 10 mg kg^{-1} citalopram combined with 0.3 mg kg^{-1} s.c. WAY100635. The same effects were found when citalopram was injected with 0.1 μg WAY100635 into the DR. Since stimulation of 5-HT$_{2B}$ receptors was recently found to cause hyperphagia (Kennett *et al*., 1997a), the results with SB206553 suggest that 5-HT$_{2C}$ receptors mediate the hypophagia caused by the combination of citalopram/WAY100653.

The fact that SB206553 did not modify the effect of citalopram alone is consistent with previous findings that hypophagia by SSRIs in food-deprived rats is affected little, if at all, by substances that block central 5-HT$_{2C}$ receptors (Grignaschi & Samanin, 1992, 1993; Lightowler *et al*., 1996). Citalopram's failure to stimulate the 5-HT$_{2C}$ receptors involved in feeding control is very likely due to the fact that by inhibiting 5-HT cell firing through increasing endogenous 5-HT in the raphe area it limits its ability to raise extracellular 5-HT in terminal regions. The combination with WAY100635 would raise extracellular 5-HT sufficiently to cause functional effects through stimulation of 5-HT$_{2C}$ receptors. It is of interest that fenfluramine, a drug whose effect on extracellular 5-HT does not depend on impulse flow in 5-HT neurons (Carboni & Di Chiara, 1989), was found to reduce food intake of food-deprived rats by a mechanism involving 5-HT$_{2C}$ receptors (Hartley *et al*., 1995). The theory that 5-HT$_{2C}$ receptors are important in the serotonergic control of feeding has recently received strong support from the finding that mice lacking 5-HT$_{2C}$ receptors are overweight and insensitive to the food-reducing effect of mCPP (Tecott *et al*., 1995).

The finding that the 5-HT$_{2B/2C}$ receptor antagonist SB206553 in one instance significantly reduced feeding apparently contradicts previous studies in which the 5-HT$_{2B/2C}$ receptor antagonist had no effect or tended to increase food intake in free feeding rats (Kennett *et al*., 1997a). The use of food-deprived vs free feeding rats may account for this discrepancy since SB200646, another 5-HT$_{2B/2C}$ receptor antagonist, tended to reduce food intake in food deprived rats (Kennett *et al*., 1994) to an extent similar to that found in the present study with SB206553, while it significantly increases feeding in slightly food-deprived rats (Kennett *et al*., 1995). Since the stimulation of 5-HT$_{2B}$ receptors induces feeding and this effect is reduced by SB206553 (Kennett *et al*., 1997a), blockade of 5-HT$_{2B}$ receptors may have contributed to its slight hypophagic effect. This is reinforced by the finding that the selective 5-HT$_{2C}$ receptor antagonist SB242084 (Kennett *et al*., 1997b) has no effect on food intake in food-deprived rats (unpublished results).

The present study has shown that the hypophagic effect of citalopram is potentiated by blocking 5-HT$_{1A}$ receptors. Only the effect of the WAY100635/citalopram combination seems to be mediated by central 5-HT$_{2C}$ receptors. It remains to clarify which receptor mechanisms mediate the effects of SSRIs on food intake when given alone. Another 5-HT receptor subtype that has been frequently involved in 5-HT-dependent anorexia is the 5-HT$_{1B}$ type (see Samanin & Grignaschi, 1996 for review). Our previous studies with fluoxetine and sertraline (Grignaschi & Samanin, 1992, 1993) and one study in which citalopram was combined with the selective 5-HT$_{1B/1D}$ receptor antagonist GR127935 (unpublished results) seem to exclude that this receptor is involved in SSRIs' hypophagia. Other 5-HT receptors not yet explored include the 5-HT$_6$ and 5-HT$_7$ subtypes for which very selective antagonists are not yet available. It could be of interest that the 5-HT$_7$ receptor is highly expressed in the hypothalamus, an area particularly important in feeding mechanisms (Gustafson *et al*., 1996).

Although further studies are necessary to clarify the specificity of the effect of the citalopram/WAY100635 combination on food intake, the present findings clearly show that a typical effect of SSRIs is potentiated by blockade of 5-HT$_{1A}$ receptors, probably related to an

enhanced effect on extracellular 5-HT. Clinical studies with 5-HT$_{1A}$ receptor antagonists are needed to confirm that they enhance the antidepressant and anorectic activities of SSRIs.

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