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An investigation of the mechanism responsible for fluoxetine-induced hypophagia in rats

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Abstract

The effect of fluoxetine on feeding in *p*-chlorophenylalanine (PCPA) pretreated rats and the nature of its interaction with 5-HT_{2C} receptors have been investigated. Animals that received 3 days PCPA (150 mg/kg i.p.) pretreatment and vehicle on the test day consumed a similar amount as control, saline pretreated animals under the test paradigm used in this study. Fluoxetine (20 and 30 mg/kg p.o.) significantly reduced food intake in PCPA and control pretreated animals to a similar extent, despite an approximately 90% reduction in the levels of brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) in the PCPA-pretreated animals. Thus, hypophagia is unlikely to be caused by inhibition of 5-HT reuptake. In the pig choroid plexus in vitro, fluoxetine and norfluoxetine inhibited specific [^{3}H] mesulergine binding with pK_I's (±S.E.M.) of 6.45 ± 0.09 (n = 4) and 6.05 ± 0.05 (n = 3), and slope factors (±S.E.M.) of 1.06 ± 0.14 and 0.99 ± 0.13, respectively. In slices of piglet choroid plexus fluoxetine (1, 10 and 33 μM) caused a rightward shift in the dose-response curve produced by 5-HT with no effect on the maximal response, and a mean pK_B of 5.94 ± 0.09. Norfluoxetine (10 μM) also produced a rightward shift in the 5-HT dose-response curve with no effect on the maximal response, and a pK_B of 6.20. Thus, both compounds acted as surmountable antagonists with no agonist efficacy at 5-HT_{2C} receptors present in choroid plexus. The hypophagic effect of fluoxetine (20 mg/kg p.o) was also unaffected by the non-specific 5-HT_{2C} receptor antagonist metergoline (2 and 5 mg/kg i.p.). These findings suggest that the hypophagic effect of fluoxetine is not likely to be dependent upon intact brain 5-hydroxytryptaminergic presynaptic function, nor is it mediated by an agonist action at the 5-HT_{2C} receptor, but by an additional, unknown mechanism.

Keywords: Fluoxetine; Hypophagia; 5-HT_{2C} receptor; (Rat); (Pig)

1. Introduction

Fluoxetine, an antidepressant that is reported to selectively inhibit the reuptake of 5-hydroxytryptamine (5-HT), reduces hunger and food intake in humans (McGuirk and Silverstone, 1990) and produces hypophagia in rats (Wong et al., 1988). Since increased 5-HT neurotransmission reduces food intake (Samanin, 1983), it has been assumed that the hypophagic effect of fluoxetine in rats is due to an increase in the extracellular levels of 5-HT. However, it has been reported that its hypophagic effect in food-deprived rats is not reversed by a variety of 5-HT receptor antagonists (Wong et al., 1988; Grignaschi and Samanin, 1992). Moreover, fluoxetine-induced hypophagia in non-deprived rats is maintained following an approximate 70% reduction in the level of brain 5-HT, produced by pre-administration of 5,7-dihydroxytryptamine (5,7-DHT) intracerebroventricularly (Grignaschi and Samanin, 1992). This is in contrast to the finding that the hypophagic effect of another selective serotonin reuptake inhibitor, sertraline, is indeed attenuated by 5,7-dihydroxytryptamine lesioning of 5-HT-containing neurones (Lucki et al., 1988; Cervo et al., 1991). Thus, unlike sertraline the hypophagic effect of fluoxetine may be independent of neuronal 5-HT. However, after 5,7-DHT administration there is an upregulation of some postsynaptic 5-HT receptors (Nelson et al., 1978; Zemlan et al., 1983), and, since 30% of rat brain 5-HT was maintained in the Grignaschi study, it cannot be excluded that the remaining

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5-HT-containing neurones were able to mediate fluoxetine-induced hypophagia.

In this study, we have further investigated the possible role of endogenous 5-HT in the hypophagic effect of fluoxetine, by examining the effects of fluoxetine in food-deprived rats following 3 days pretreatment with *p*-chlorophenylalanine (PCPA) – a 5-HT synthesis inhibitor reported to reduce brain 5-HT levels by approximately 90% after three daily doses (Dourish et al., 1986). After an assessment of the effect of fluoxetine on feeding in PCPA-pretreated animals, the level of 5-HT and its metabolite, 5-hydroxyindole acetic acid (5-HIAA), in their whole brains was measured.

Fluoxetine and its metabolite, norfluoxetine have an appreciable affinity for the 5-HT_{2C} receptor subtype (Wong et al., 1991, 1993), but whether they act as agonists or antagonists at this site is unknown. Since 5-HT_{2C} receptor activation produces hypophagia in rats (Kennett and Curzon, 1988, 1991), an agonist action at this receptor subtype by either fluoxetine or its metabolite may contribute to, or be responsible for, its hypophagic effect in normal and/or 5,7-DHT-pretreated animals. In an attempt to investigate the nature of the interaction between fluoxetine and norfluoxetine and the 5-HT_{2C} receptor subtype, experiments have been carried out, in vitro, to examine whether they act as agonists or antagonists at 5-HT_{2C} receptors present in the pig choroid plexus. In order to further investigate the possible role of 5-HT_{2C} receptors in vivo, we have conducted experiments to determine whether metergoline (a non-specific 5-HT_{2C} receptor antagonist) affects fluoxetine-induced hypophagia.

2. Materials and methods

2.1. Feeding experiments

2.1.1. Food intake

Male Sprague-Dawley rats (Charles River) (250–300 g) were administered PCPA (methyl ester) (150 mg/kg i.p.–2 ml/kg) or $vehicle_1$ (0.9% saline) daily for 3 days. On day 3, the animals were housed singly and food-deprived for 23 h pre-test. On day 4, animals from each pre-treatment group were given fluoxetine (20 or 30 mg/kg p.o. – 1 ml/kg) or $vehicle_2$ (1% methyl cellulose solution) ($n = 17–20$/group). 1 h later, approximately 150 g of CRMX rat diet (SDS Whitam) was placed into each animal's food hopper. After a further 4 h, the food remaining in the hoppers was weighed, and the amount consumed by each rat determined.

In experiments carried out to investigate the effects of metergoline, similar animals were also housed singly and food-deprived for 23 h pre-test. On the test day, animals ($n = 20$/group) were dosed with fluoxetine (20 mg/kg p.o. – 1 ml/kg) or $vehicle_2$ and, 30 min later, with metergoline (2 or 5 mg/kg i.p. – 1 ml/kg) or $vehicle_1$. After a further 30 min, approximately 150 g of CRMX rat diet (SDS Whitam) was placed into each animal's food hopper, and the amount consumed by each rat during the following hour was determined.

2.1.2. Measurement of brain 5-HT and 5-HIAA levels

Immediately after testing, 10 animals from each pretreated group that were dosed with vehicle on the test day, were sacrificed and their brains were removed. The brains were frozen and stored at −80°C. Upon thawing, the whole brains were homogenised in 0.4 N $HClO_4$ containing 0.1% Na bisulphate, 0.1% cysteine and 0.01% EDTA. Levels of supernatant 5-HT and 5-HIAA were determined using HPLC with electrochemical detection (Hutson et al., 1989).

2.2. Assessment of 5-HT_{2C} receptor activity

Pig brains were obtained from a local slaughterhouse and placed on ice immediately after death. Choroid plexus was carefully removed from the ventricular system, and sieved through nylon mesh (250 μm) prior to homogenisation.

2.2.1. 5-HT_{2C} receptor affinity

The affinity of fluoxetine for the 5-HT_{2C} receptor was determined by measuring its displacement of ^{3}[H]mesulergine binding, using the method described by Pazos et al. (1984), with the exceptions that 3 nM spiperone was present, the filters were pre-soaked in polyethyleneimine (0.01%), and mianserin (1 μM) was used to define non-specific binding. Binding parameters for drug competition studies were calculated using the 4-parameter logistic function according to De Lean et al. (1978) and IC_{50} converted to K_1 values using the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

2.2.2. Measurement of 5-HT_{2C} receptor-activated inositol phosphate accumulation

Inositol phosphate accumulation in piglet choroid plexi was measured as described previously (Brown et al., 1991). Briefly, once removed, the choroid plexi were cross chopped on a McIlwain tissue chopper set at 300 μm. The slices were washed twice in gassed (95% O_2-5% CO_2) Na^+-Krebs at 37°C and then incubated with [^{3}H]*myo*-inositol (10–20 Ci/mmol; stock solution dried under nitrogen and redissolved in Na^+-Krebs) for 1.5–2 h at 37°C with constant gassing. After this incubation, the slices were washed 3 times. The slices were finally resuspended in an appropriate volume of Li^+-Krebs containing 100 μM pargyline, 6 μM cocaine and 0.2 mM ascorbic acid. Incubations were carried out in Beckman Biovials in Li^+-Krebs for 1 h at 37°C in a shaking water bath. The total incubation volume was 300 μl. Drugs were added in a total volume of 20 μl. 5-HT was dissolved in 2 mM ascorbic

acid and was added in 10 μl. Drugs were dissolved in Li^+-Krebs containing 0.2 mM ascorbic acid and added in 10 μl. Incubations were started by addition to each vial of 280 μl of the slice suspension. The vial contents were gassed/mixed with a jet of 95% O_2-5% CO_2 and sealed. Incubations were terminated by addition of chloroform-methanol and the inositol phosphates separated (Minchin and Wood, 1986). pK_B values were calculated for the antagonists, defined as $-\log_{10}K_B$, where $K_B = [\text{antagonist}]/DR - 1$. DR is the dose ratio (the factor by which the concentration of the agonist has to be increased in the presence of the antagonist to obtain an effect identical to that observed in the absence of the antagonist). The antagonist concentration is expressed in M and K_B is the apparent dissociation constant.

2.3. Drugs

Fluoxetine hydrochloride was extracted from Eli Lilly 'Prozac' tablets by SmithKline Beecham Pharmaceuticals; norfluoxetine was made by SmithKline Beecham Pharmaceuticals; PCPA, polyethyleneimine, pargyline and cocaine were obtained from Sigma; EDTA and ascorbic acid were obtained from BDH, Merck, Merck House, Poole, Dorset, UK; [^{3}H]mesulergine and [^{3}H]*myo*-inositol were obtained from Amersham International, Amersham Place, Little Chalfont, Bucks, UK; spiperone, metergoline and mianserin were obtained from RBI, Semat Technical (UK), Hatfield Road, St. Albans, Herts, UK.

2.4. Statistics

The results from the food intake experiments were analyzed by Newman-Keuls test following a significant two way analysis of variance. The change in brain levels of 5-HT and 5-HIAA was tested for significance by Student's *t*-type test.

3. Results

3.1. The effects of PCPA on fluoxetine-induced hypophagia and brain levels of 5-HT and 5-HIAA

In saline-pretreated animals fluoxetine, at 20 and 30 mg/kg, significantly ($P < 0.01$) reduced mean (±S.D.) food intake from 7.77 ± 1.27 g ($n = 18$) to 4.04 ± 2.40 g ($n = 17$) and 5.52 ± 1.76 g ($n = 17$), respectively. Animals that received PCPA pretreatment and vehicle$_2$ on the test day consumed a similar amount to the control saline-pretreated animals (7.36 ± 1.84, $n = 17$). In PCPA-pretreated animals, fluoxetine, at 20 and 30 mg/kg, significantly ($P < 0.01$) reduced food intake to

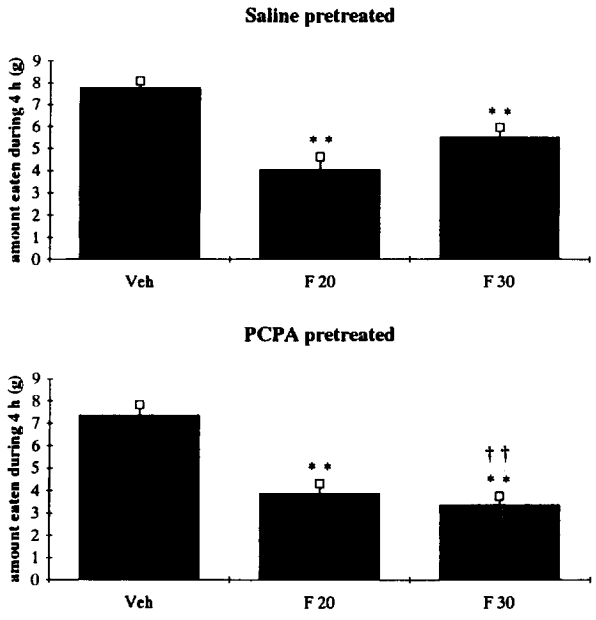

Fig. 1. The effect of fluoxetine (20 and 30 mg/kg p.o.) on food intake (4 h) in PCPA (150 mg/kg i.p.) pretreated (daily for 3 days), food deprived (23 h) rats. Significantly different from appropriate vehicle treated group $^{**}P < 0.01$, from corresponding saline pretreated group $^{††}P < 0.01$ by the Newman-Keuls test. Verticle bars represent S.E.M., 17–20 animals/group.

3.87 ± 1.86 ($n = 18$) and 3.37 ± 1.70 ($n = 20$), respectively. The reduction in the amount eaten following fluoxetine at 30 mg/kg in PCPA-pretreated animals was significantly greater than the reduction produced by the same dose in saline-pretreated animals. Significant F values: effect of PCPA, $F = 6.6$ df 1, 101, $P < 0.05$; effect of fluoxetine, $F = 40.8$, df 2, 101, $P < 0.01$; interaction of fluoxetine with mCPP, $F = 12.6$, df 2, 101, $P < 0.01$ (Fig. 1).

PCPA pretreatment caused an approximately 90% reduction in brain levels of 5-HT and 5-HIAA (Table 1).

Metergoline alone, at each dose tested, did not significantly affect the amount of food eaten, nor did it affect the significant reduction produced by fluoxetine at 20 mg/kg (Table 2). F values: effect of 2 mg/kg metergoline, $F = 1.0$, df 1, 76, not significant, effect of fluoxetine $F = 15.6$, df 1,76, $P < 0.01$, interaction of fluoxetine with metergoline $F = 0.3$, df 1, 76, not signif-

Table 1
The effect of PCPA (150 mg/kg i.p., three daily doses) on whole brain 5-HT and 5-HIAA

Pretreatment (daily for 3 days)	Level of 5-HT (ng/g)	Level of 5-HIAA (ng/g)
Saline (i.p.)	316.7 ± 20.3	251.1 ± 9.7
PCPA (150 mg/kg i.p.)	41.4 ± 3.0 [a] (−87%)	20.6 ± 2.0 [a] (−92%)

Values are means ± S.E.M. ($n = 10$). [a] $P < 0.001$ different from saline-pretreated animals by Student's *t*-test.

Table 2
The effect of metergoline (2 and 5 mg/kg i.p.) on fluoxetine (20 mg/kg p.o.)-induced hypophagia

p.o. pretreatment 1 h pretest	i.p. treatment 30 min pretest	Amount eaten during 1 h (g)
$Vehicle_2$	$Vehicle_1$	5.3 ± 0.4
Fluoxetine	$Vehicle_1$	3.1 ± 0.3 [b]
$Vehicle_2$	Metergoline (2 mg/kg)	4.6 ± 0.3
Fluoxetine	Metergoline (2 mg/kg)	3.2 ± 0.3 [b]
$Vehicle_2$	$Vehicle_1$	4.3 ± 0.3
Fluoxetine	$Vehicle_1$	2.9 ± 0.2 [b]
$Vehicle_2$	Metergoline (5 mg/kg)	3.7 ± 0.2
Fluoxetine	Metergoline (5 mg/kg)	2.8 ± 0.3 [a]

Values are means ± S.E.M. ($n = 17$–20). [a,b] $P < 0.05$, 0.01 different from relevant control + $vehicle_2$-treated group by the Newman–Keuls test.

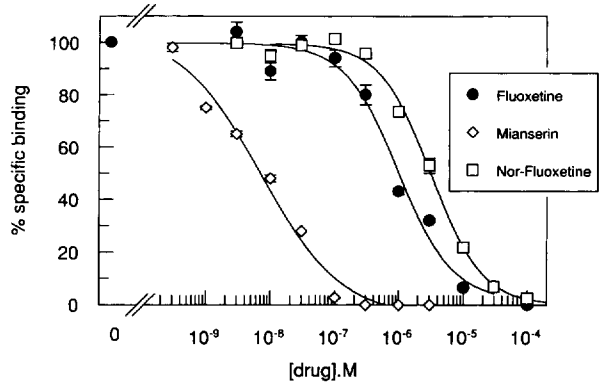

Fig. 3. Example of the effect of fluoxetine (33 μM) on 5-HT-stimulated inositol phosphate production in piglet choroid plexus in vitro. ○, 5-HT; ●, 5-HT + fluoxetine (33 μM).

Table 3
Inhibition of specific [^{3}H]mesulergine binding to the 5-HT_{2C} receptor by fluoxetine

	pK_I (n)	Slope factor
Fluoxetine	6.45 ± 0.09 (4)	1.06 ± 0.14
Norfluoxetine	6.05 ± 0.05 (3)	0.99 ± 0.13
Mianserin	8.74 ± 0.03 (3)	0.92 ± 0.14

Values are means ± S.E.M. ($n = 4$).

icant; effect of 5 mg/kg metergoline, $F = 2.4$, df 1, 65, not significant, effect of fluoxetine $F = 21.9$, df 1, 65, $P < 0.01$, interaction of fluoxetine with metergoline $F = 1.3$, df, 1, 65 not significant.

3.2. Interaction of fluoxetine with 5-HT_{2C} receptors in the pig choroid plexus

Fluoxetine and norfluoxetine inhibited specific [^{3}H]mesulergine binding, in the pig choroid plexus, with pK_Is of 6.45 ± 0.09 ($n = 4$) and 6.05 ± 0.05 ($n = 3$), and slope factors of 1.06 ± 0.14 and 0.99 ± 0.13, respectively (Table 3; Fig. 2). In slices of piglet choroid plexus, 5-HT caused a 10-fold increase in inositol phosphate accumulation with an EC_{50} of 45 nM ± 3 ($n = 3$). Fluoxetine (1, 10 and 33 μM) had no effect on inositol phosphate production, but caused a rightward shift in the dose-response curve produced by 5-HT with no effect on the maximal response, and, from three experiments, a mean pK_B of 5.94 ± 0.09 (Fig. 3 for an example). Norfluoxetine (10 μM) also had no effect alone and produced a rightward shift in the 5-HT dose-response curve with no effect on the maximal response, giving a pK_B of 6.20. Neither mesulergine nor mianserin stimulated inositol phosphate production (10–100 μM), but also antagonised the effects of 5-HT with no effect on the maximal response, giving mean pK_B's of 8.83 ± 0.14 ($n = 6$) and 8.12 ± 0.19 ($n = 6$), respectively.

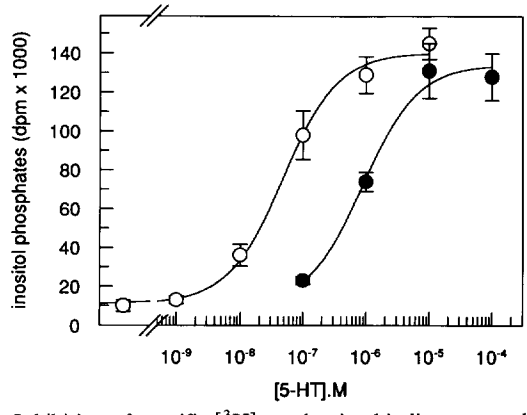

Fig. 2. Inhibition of specific [^{3}H]mesulergine binding to membranes of pig choroid plexus by mianserin, fluoxetine and norfluoxetine. Data are means ± S.E.M. ($n = 3$).

4. Discussion

It has been assumed that the hypophagic effect of fluoxetine is mediated via its ability to inhibit the reuptake of 5-HT, and thereby increase the synaptic availability of this neurotransmitter. However, the results from this study demonstrate that fluoxetine-induced hypophagia is maintained in rats following an approximately 90% reduction in brain 5-HT and 5-HIAA levels, and is therefore probably 5-HT independent. This conclusion is consistent with the failure of 5-HT-containing neurone lesioning by 5,7-dihydroxytryptamine to alter fluoxetine-induced hypophagia in rats (Grignaschi and Samanin, 1992). However, in the former study approximately 30% of brain 5-HT remained, which may have been sufficient for a 5-HT-mediated hypophagic response. Furthermore, results from an in vivo microdialysis study by Sharp et al. (1989), demonstrated that although intracerebroven-

tricular 5,7-DHT administration reduced the level of hippocampal 5-HT by 90%, the reduction in extracellular 5-HT (2h after probe implantation), was only 57%. Thus, the 70% reduction in brain 5-HT reported in the study of Grignaschi and Samanin (1992) may be an overestimate of the reduction in synaptic 5-HT availability. In contrast, results from another in vivo microdialysis study have demonstrated that following PCPA pretreatment the amount of 5-HT release from neurones is proportional to its depletion (O'Connell et al., 1991). Therefore, the 90% reduction in overall brain 5-HT observed in our study can be considered as representing a similar reduction in synaptically released 5-HT. The maintained hypophagia following a reduction in brain 5-HT by PCPA administration is therefore stronger evidence against increased synaptic 5-HT availability as being the mechanism responsible. However, it must be borne in mind that a small percentage of 5-HT-like immunoreactive fibres in the anterior or lateral hypothalamus are resistant to PCPA administration (Tohyama et al., 1988). Thus, an increase in the synaptic availability of 5-HT by fluoxetine in this region cannot be ruled out as mediating its hypophagic effect.

Concerning the interaction of fluoxetine with 5-HT_{2C} receptors, we have substantiated earlier reports that fluoxetine and its metabolite, norfluoxetine, have appreciable affinities for this receptor subtype in bovine brain (Wong et al., 1991, 1993), in a second animal species (porcine). The small difference in affinity estimates from the binding and functional experiments seen in this study may represent methodological differences, but are, nevertheless, in good agreement. Since activation of 5-HT_{2C} receptors is associated with hypophagia in rats (Kennett and Curzon, 1988, 1991), an agonist action by fluoxetine and/or norfluoxetine at this site may have explained the maintenance of hypophagia in PCPA-pretreated animals. Yet in the pig choroid plexus, a functional model of 5-HT_{2C} receptor activation (Brown et al, 1991), our results reveal that fluoxetine and norfluoxetine act as competitive antagonists and have no intrinsic activity at this 5-HT receptor subtype. However, it is conceivable that fluoxetine and/or norfluoxetine posses some intrinsic activity, and, due to an apparent low receptor reserve in the pig choroid plexus (Brown et al., 1991), act as competitive antagonists in this tissue, but may demonstrate agonist activity in a tissue that with a greater reserve of 5-HT_{2C} receptors. Furthermore, it is possible that the pig choroid plexus is not appropriate when assessing intrinsic activity of agents to be behaviourally tested in rats. Against these caveats is our observation that fluoxetine-induced hypophagia is not reduced by metergoline at a dose that reverses known 5-HT_{2C} and/or 5-HT_{2B} receptor-mediated hypophagia (Samanin et al., 1979; Kennett et al., 1994), a finding that is in keeping with those of other workers (Wong et al., 1988; Grignaschi and Samanin, 1992).

Interestingly, fluoxetine (30 mg/kg) reduced feeding significantly more in PCPA-pretreated, than in vehicle-pretreated animals (−54 vs. −29%). Since PCPA administration upregulates 5-HT_{2C}, but not 5-HT_{1A} or 5-HT_{2A}, receptor-mediated responses (Berendsen et al., 1991), an increase in fluoxetine-induced hypophagia might indeed be expected in PCPA-pretreated animals if it and/or its major metabolite act as direct agonists at this receptor subtype. However, fluoxetine and/or norfluoxetine may interact directly with other, as yet uninvestigated, 5-HT receptor subtypes, the function of which may also increase following PCPA administration. Alternatively, fluoxetine and/or norfluoxetine may interact with non-5-HT systems that are also unregulated following PCPA pretreatment, albeit indirectly. For example, somatostatin and neuropeptide Y concentrations in the rat brain are markedly elevated following PCPA administration (Kakigi and Maeda, 1992).

When considering other possible mechanisms, a 5-HT-independent action of fluoxetine, that may influence feeding control, is its ability to stimulate glycogenolysis in astrocytes, in vitro (Zhang et al., 1993). Although attributed to direct stimulation of 5-HT_{2C} receptors, the high concentrations of antagonists used by Zhang and coworkers (100 μM mesulergine, for example) make this conclusion unlikely. Although possibly not mediated by activation of 5-HT_{2C} receptors, the stimulation of glycogenolysis by fluoxetine in astrocytes is intriguing, firstly because it occurs in the absence of 5-HT, and, secondly, because it occurs at concentrations of fluoxetine that may be therapeutically relevant (Zhang et al., 1993).

As outlined in the Introduction, it is important to note that not all selective serotonin reuptake inhibitors have the same hypophagic profile as fluoxetine. For example, the hypophagic effect of sertraline is attenuated by 5,7-dihydroxytryptamine lesioning of 5-HT-containing neurones (Lucki et al., 1988; Cervo et al., 1991). Thus, unlike fluoxetine, the anorectic effect of sertraline seems to be dependent upon the synaptic availability of 5-HT.

One other property not shared by all selective serotonin reuptake inhibitors seems to be their influence on brain neuropeptide Y (NPY). Single and repeated administration of fluoxetine causes a reduction in the level of neuropeptide Y (NPY) in the paraventricular nucleus and lateral hypothalamic area of the rat brain (Dryden et al., 1994). However, repeated administration of another selective serotonin reuptake inhibitor, zimelidine, has no such effect on hypothalmic NPY, and even increases its level in the frontal cortex (Heilig et al., 1988). Also, although the hypothalamus was not studied, repeated clomipramine administration has no

effect on NPY level in the rat frontal cortex and hippocampus (Bellman and Sperk, 1993). Since NPY administration into the paraventricular nucleus stimulates feeding (Stanley and Leibowitz, 1984), its reduction by fluoxetine by an, as yet, unknown mechanism may account for fluoxetine-hypophagia.

Clearly, the findings from this study do not reveal the mechanism of fluoxetine-induced hypophagia. However, they support a mechanism(s) other than an increase in the extracellular availability of 5-HT or direct stimulation of 5-HT_{2C} receptors. Curiously, this is the same conclusion reached by Gibson et al. (1993) for *d*-fenfluramine, another inhibitor of feeding, previously thought to act by increasing the synaptic availability of 5-HT, and which also causes a reduction in NPY level in the ventromedial and dorsomedial nuclei in the lateral hypothalamus (Rogers et al., 1991).

References

Bellman, R. and G. Sperk, 1993, Effects of antidepressant drug treatment on levels of NPY or prepro-NPY-mRNA in the rat brain, Neurochem. Int. 22, 183.

Berendsen, H.H., C.L. Broekkamp and A.M. Delft, 1991, Depletion of brain serotonin differently affects behaviours induced by 5-HT_{1A}, 5-HT_{1C} and 5-HT_2 receptor activation in rats, Behav. Neural. Biol. 55, 214.

Brown, A.M., T.L. Patch and A.J. Kauman, 1991, The antimigraine drugs ergotamine and dihydroergotamine are potent 5-HT_{1C} receptor agonists in piglet choroid plexus, Br. J. Pharmacol. 104, 45.

Cervo, L., G. Grignaschi, C. Rossi and R. Samanin, 1991, Role of central serotonergic neurons in the effect of sertraline in rats in the forced swimming test, Eur. J. Pharmacol. 196, 217.

Cheng, Y. and W.H. Prusoff, 1973, Relationship between the inhibition constant (K_1) and the constant of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction, Biochem. Pharmacol. 22, 3099.

DeLean, A., J. Stadel and R.J. Lefkowitz 1980, A ternary complex model explains the agonist specific binding properties of the adenylate cyclase-coupled β-adrenergic receptor, J. Biol. Chem. 255, 7108.

Dourish, C.T., P.H. Hutson and G. Curzon, 1986, Para-chlorophenylalanine prevents feeding induced by the serotonin agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), Psychopharmacology 89, 467.

Dryden, S., H.D. McCarthy, H.M. Frankish, A. Kilpatrick and G. Williams, 1994, Does NPY mediate the anorectic effect of serotonin? The serotonergic agent fluoxetine causes specific changes in NPY concentrations in appetite regulating hypothalamic nuclei in the rat, Clin. Sci. 86 (2), 43P.

Gibson, E.L., A.J. Kennedy and G. Curzon, 1993, *d*-Fenfluramine- and *d*-norfenfluramine-induced hypophagia: differential mechanisms and involvement of postsynaptic 5-HT receptors, Eur. J. Pharmacol. 242, 83.

Grignaschi, G. and R. Samanin, 1992, Role of serotonin and catecholamines in brain in the feeding suppressant effect of fluoxetine, Neuropharmacology 31, 445.

Heilig, M., C. Wahlestedt, R. Ekman and E. Widerlov, 1988, Antidepressant drugs increase the concentration of neuropeptide Y (NPY)-like immunoreactivity in the rat brain, Eur. J. Pharmacol. 147, 465.

Hutson, P.H., G.S. Sarna, M.T. O'Connell and G. Curzon, 1989, Hippocampal 5-HT synthesis and release in vivo is decreased by infusion of 8-OHDPAT into the nucleus raphe dorsalis, Neurosci. Lett. 100, 276.

Kakigi, T. and K. Maeda, 1992, Effect of serotonergic agents on regional concentrations of somatostatin- and neuropeptide Y-like immunoreactivities in rat brain, Brain. Res. 599, 45.

Kennett, G.A. and G. Curzon, 1988, Evidence that hypophagia induced by mCPP and TFMPP requires 5-HT_{1B} and 5-HT_{1C} receptors; hypophagia induced by RU 24969 only requires 5-HT_{1B} receptors, Psychopharmacology 95, 93.

Kennett, G.A. and G. Curzon, 1991, Potencies of antagonists indicate that 5-HT_{1C} receptors mediate1-2(chlorophenyl)piperazine-induced hypophagia, Br. J. Pharmacol. 103, 2016.

Kennett, G.A., M.D. Wood, A. Glen, S. Grewel, I. Forbes, A. Gadre and T.P. Blackburn, 1994, In vivo properties of SB 200646A, a selective 5-$HT_{2B/2C}$ receptor antagonist, Br. J. Pharmacol. 111, 797.

Lucki, I., M.S. Kreider and K.J. Simansky, 1988, Reduction of feeding behaviour by the serotonin uptake inhibitor sertraline, Psychopharmacology 96, 289.

McGuirk, J. and T. Silverstone, 1990, The effect of the 5-HT re-uptake inhibitor fluoxetine on food intake and body weight in healthy male subjects, J. Obesity 14, 361.

Minchin, M.C.W. and M.D. Wood, 1986, A convenient procedure for the assay of [^{3}H]-labelled inositol complex, Br. J. Pharmacol. 89, 784P.

Nelson, D.L., A. Herbert, S. Bourgoin, J. Glowinski and M. Hamon, 1978, Characteristics of central 5-HT receptors and their adaptive changes following intracerebral 5,7-dihydroxytryptamine administration in the rat, Mol. Pharmacol. 14, 983–995.

O'Connell, M.T., C.M. Portas, G.S. Sarna and G. Curzon, 1991, Effect of *p*-chlorophenylalanine on release of 5-HT from the rat frontal cortex in vivo, Br. J. Pharmacol. 102, 831.

Pazos, A., D. Hoyer and J.M. Palacios, 1984, The binding of serotonergic ligands to the porcine choroid plexus: characterization of a new type of serotonin recognition site, Eur. J. Pharmacol. 106, 539.

Rogers, P., P.E. McKibbin and G. Williams, 1991, Acute fenfluramine administration reduces neuropeptide Y concentrations in specific hypothalamic regions of the rat: possible implications for the anorectic effect of fenfluramine, Peptides 12, 251.

Samanin, R., 1983, Drugs affecting serotonin and feeding, in: Biochemical Pharmacology of Obesity, eds. P.B. Curtis-Prior (Elsevier, Amsterdam) p. 339.

Samanin, R., T. Mennini, A. Ferraris, C. Bendotti, F. Borsini and S. Garattini, 1979, *m*-Chlorophenylpiperazine: a central serotonin agonist causing powerful anorexia in rats, Naunyn-Schmied. Arch. Pharmacol. 308, 159.

Sharp, T., S.R. Bramwell, D. Clark and D.G. Grahame-Smith, 1989, In vivo measurement of extracellular 5-hydroxytryptamine in hippocampus of the anaesthetized rat using microdialysis: changes in relation to 5-hydroxytryptamine neuronal activity, J. Neurochem. 53, 234.

Stanley, B.G. and S.F. Liebowitz, 1984, Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus, Life Sci. 35, 2635.

Tohyama, I., M. Kameyama and H. Kimura, 1988, Quantitative morphometric analysis of two types of serotonin-immunoreactive nerve fibres differentially responding to *p*-chlorophenylalanine treatment in the rat brain, Neuroscience 26, 971.

Wong, D.T., L.R. Reid and P.G. Threlkeld, 1988, Suppression of food intake in rats by fluoxetine: comparison of enantiomers and effects of serotonin antagonists, Pharmacol. Biochem. Behav. 31, 475.

Wong, D.T., P.G. Threlkeld and D.W. Robertson, 1991, Affinities of fluoxetine, its enantiomers, and other inhibitors of serotonin

uptake for subtypes of serotonin receptors, Neuropsychopharmacology 5, 43.

Wong, D.T., F.P. Bymaster, L.R. Reid, D.A. Mayle, J.H. Krushinski and D.W. Robertson, 1993, Norfluoxetine enantiomers as inhibitors of serotonin uptake in rat brain, Neuropsychopharmacol. 8, 337.

Zemlan, F.P., L.-M. Kow and R.W. Pfaff, 1983, Spinal serotonin (5-HT) receptor subtypes and nociception, J. Pharmacol. Exp. Ther. 226, 477.

Zhang, X.L. Peng, Y. Chen and L. Hertz, 1993, Stimulation of glycogenolysis in astrocytes by fluoxetine, an antidepressant acting like 5-HT, NeuroReport 4, 1235.