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Serotonergic and histaminergic mechanisms involved in intralipid drinking?

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Abstract

Some newer antipsychotic agents are associated with weight gain in humans and a hyperphagic response to intralipid solutions in rodents. To examine the possible contribution of serotonin (5-HT) and histamine (H) receptor blockade in antipsychotic-associated hyperphagia, rats were trained to drink a palatable, high-calorie fat emulsion (10% intralipid) during 30-min sessions and were tested following pretreatment with mepyramine (H_1 receptor antagonist), metergoline (5-$HT_{1/2}$ receptor antagonist), cyproheptadine (H_1 and 5-$HT_{2A/2B/2C}$ and muscarinic receptor antagonist), SB 242084 (5-HT_{2C} receptor antagonist) and an SB 242084–mepyramine combination. Total intake and ingestive behaviour microstructure were measured. Mepyramine (10 mg/kg) reduced intake, as did metergoline (3.0 mg/kg). Cyproheptadine (0.1–1.0 mg/kg) increased intake and microstructural analysis suggests that this was due to increased numbers of clusters of licking. SB 242084 (3 mg/kg) reduced intake, either when administered alone, or in combination with mepyramine (1 mg/kg). In conclusion, simple antagonism of either H_1 (mepyramine) or 5-$HT_{1/2}$ receptors (metergoline) alone was not sufficient to increase intake. Furthermore, combined blockade of H_1 and 5-HT_{2C} receptors (SB 242084 and mepyramine) was also insufficient to produce hyperphagia. Conversely, simultaneous blockade of H_1, 5-$HT_{2A/2C}$ and muscarinic receptors (cyproheptadine) led to a substantial hyperphagia and pattern of ingestive behaviour that was similar to that previously observed with some newer antipsychotic agents.

Keywords: Mepyramine; Metergoline; Cyproheptadine; SB 242084; Serotonin; Histamine; Antipsychotic; Rat; Hyperphagia

1. Introduction

Treatment with atypical antipsychotics, such as clozapine and olanzapine, are associated with weight gain and obesity, in humans. For example, in a recent meta-analysis, Allison et al. (1999) found that the atypical antipsychotics clozapine, olanzapine and risperidone were associated with weight gains of 4.45, 4.15 and 2.10 kg, respectively, following 10 weeks of treatment.

The antiserotonergic and antihistaminergic properties of atypical antipsychotic drugs may account for the weight gain associated with chronic treatment (Stanton, 1995; Baptista, 1999; Casey and Zorn, 2001). Both serotonin (5-HT) and histamine (H) have well-established inhibitory roles in the central control of feeding (e.g., Blundell, 1977; Dourish, 1995; Clineschmidt and Lottie, 1973; Orthen-Gambill, 1988). Blockade of either 5-HT or H receptors have been shown to increase food intake in rats. For instance, the 5-HT antagonists methysergide, metergoline and ritanserin have all been demonstrated to produce hyperphagia in maximally satiated rats (Fletcher, 1988). Antihistaminergic compounds, such as doxepin (Orthen-Gambill, 1988) and promazine (Orthen-Gambill and Salomon, 1990), have also been shown to increase food intake in rats.

Of particular interest are the 5-HT_{2C} and H_1 receptor subtypes, at which many atypical antipsychotics act as antagonists, and which are also associated with changes in feeding and body weight, following pharmacological manipulation. For example, the 5-HT_{2C} receptor agonist Ro 60-0175 has been shown to reduce food intake in rats (Martin et al., 1998; Clifton et al., 2000), and 5-HT_{2C} receptor knockout mice display hyperphagia and obesity (Tecott et al., 1995). The H_1 receptor antagonist mepyramine attenuates the reduction of feeding induced by the H-breakdown-

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enzyme-inhibitor metoprine (Lecklin and Tuomisto, 1998). The reduction of feeding induced by intracerebroventricular H infusion is also attenuated by pretreatment with mepyramine (Lecklin et al., 1998). Interestingly, a retrospective review of the clinical records of patients taking clozapine, olanzapine, sertindole, risperidone and haloperidol found that weight gain correlated most closely with the relative H_1 receptor affinities of those compounds, rather than 5-HT_{2C} receptor affinities (Wirshing et al., 1999). A similar finding has recently been reported by Kroeze et al. (2003) in which H_1 receptor affinity was found to be strongly correlated with short-term weight gain across a range of typical and atypical antipsychotic compounds.

Hyperphagia has also been reported in rodents following antipsychotic drug treatment. Clozapine and risperidone increased food intake after 4 h, and olanzapine increased food intake after 24 h in nondeprived rats (Benvenga and Leander, 1997). Similarly, chlorpromazine, haloperidol, clozapine, olanzapine, risperidone and sulpiride have all been reported to increase food intake in nondeprived female mice over a time period ranging from 30 min to 2–5 h (Kaur and Kulkarni, 2002). Previously, we have reported that acute administrations of clozapine, olanzapine (Hartfield et al., 2001, 2002, 2003) and quetiapine (Hartfield et al., 2002) produce increased intake in a fat (10% intralipid)-drinking paradigm.

Here, we provide a systematic investigation of the possible neurochemical mechanisms that may be responsible for atypical antipsychotic-associated hyperphagia in rats, using the fat-drinking (intralipid) paradigm we have reported previously (Hartfield et al., 2001, 2002, 2003). Specifically, we examine whether intralipid hyperphagia can be produced by either H_1 or 5-HT receptor antagonism, or by a combination of H_1 and 5-HT receptor blockade. The following compounds were used: mepyramine (H_1 antagonist), metergoline (5-$HT_{1/2}$ antagonist), cyproheptadine (mixed H_1, 5-$HT_{2A/2B/2C}$ and muscarinic antagonist), a combination of mepyramine and SB 242084 (a selective 5-HT_{2C} antagonist) treatments and SB 242084 treatment alone. In addition to the measures of overall intake, we also examined the fine microstructural components of drinking behaviour that can provide insight into the behavioural processes underlying ingestion and provide more meaningful results than measuring gross intake alone.

2. Materials and methods

2.1. Subjects

Four groups of 12 male Lister-hooded rats (Harlan, UK and University of Sussex colony), weighing between 210 and 310 g at the beginning of each experiment, were used. All rats were housed in groups of three in large solid-bottomed polyethylene cages (RB2, North Kent Plastics, UK).

Animals were housed in a holding room with a 12-h light–dark cycle (lights off: 1700 h) maintained at 21–22 °C and 40–60% relative humidity. Testing took place in the light part of the photoperiod. Food (standard laboratory chow) and water was freely available throughout the experiment, except for during the drinking test sessions. All protocols were in accordance with the Animals (Scientific Procedures) Act 1986, following approval by the University of Sussex Ethical Committee.

2.2. Drugs

Mepyramine maleate (Sigma/RBI, Poole, UK) was dissolved in 0.9% saline. Metergoline phenylmethylester (Sigma/RBI) was dissolved in a vehicle of 0.5% ascorbic acid and sonicated for 15 min. Cyproheptadine hydrochloride (Sigma/RBI) was dissolved in distilled water and sonicated for 5 min. SB 242084 (synthesised by Eli Lilly, Windlesham, UK) was dissolved in 20% of final volume of PEG400 (polyethylene glycol, Sigma/RBI), sonicated for 30 min and diluted to final volume with 10% cyclodextrin [(2-hydroxypropyl)-β-cyclodextrin, Fluka, UK].

Mepyramine (1.0, 3.0 and 10 mg/kg), metergoline (0.3, 1.0 and 3.0 mg/kg), cyproheptadine (0.1, 0.3 and 1.0 mg/kg) and their vehicles were all administered via the intraperitoneal route (injection volume of 1 ml/kg), 30 min prior to the drinking-test sessions. SB 242084 (3 mg/kg) or its vehicle was administered 60 min prior to testing and was followed by mepyramine (1 mg/kg) or saline administration, 30 min before testing. Both SB 242084 and mepyramine were administered via the intraperitoneal route at an injection volume of 1 ml/kg. Pilot data from a further experiment that would have investigated a combination of metergoline (1 mg/kg) and mepyramine (1 mg/kg) suggested high levels of sedation and flattened body posture with reduced drinking, and was discontinued after one test day.

Doses chosen for each drug were in accordance with previous studies [mepyramine (Lecklin and Tuomisto, 1998), metergoline (Vickers et al., 2001) and cyproheptadine (Orthen-Gambill, 1988)]. In the mepyramine–SB 242084 combination experiment, the dose of SB 242084 (3 mg/kg) was chosen as it has been shown to significantly attenuate hypophagia induced by both *d*-fenfluramine and *d*-norfenfluramine (Vickers et al., 2001). The mepyramine dose of 1 mg/kg was considered the most suitable dose for the combination experiment, as indicated by the mepyramine dose–response experiment.

2.3. Procedures

The rats were moved to drinking cages (modified RB3 cages, North Kent Plastics) each day for a 30-min testing session and then placed back in their group cages. Animals were habituated to daily presentation of a 10% intralipid (Fresenius Kabi, Warrington, UK) emulsion in the drinking cages for 14 days, prior to starting the experiment. Intra-

lipid is supplied in a 20% commercial preparation (2 kcal/ml) and to obtain a 10% intralipid emulsion (1.0 kcal/ml), the refrigerated supply of 20% preparation was diluted each day with fresh tap water. Intralipid consists of purified soybean oil and egg phospholipids and glycerol, and it emulsifies easily, which makes it useful in drinking studies.

Forty-eight male rats were allocated into four groups of 12, and received mepyramine, metergoline, cyproheptadine or SB 242084 with mepyramine drug administrations only. Each animal acted as its own control and received each drug dose (total of three drug administrations) and vehicle in a counterbalanced design with treatments separated by a minimum of 48 h. The rats were moved to the drinking cages, immediately prior to the 30-min test session each day. The drinking cages and procedure are similar to that already described (Lee and Clifton, 1992; Ward et al., 2000), in that the test cages are standard solid-bottomed cages having a stainless steel drinking spout attached to one side wall. Intralipid emulsion was delivered from a reservoir to the spout by a peristaltic pump via polythene tubing. A microprocessor system controlled pump activation by detecting licks of the spout and switching the pump on for 0.5 s. The number of licks and pump activations were both noted independently by the recording software to a resolution of 10 ms. The pumps delivered 1.40 g of emulsion per minute (or 11.7 mg per 0.5 s pump activation), thus allowing pump activation to be used as a measure of consumption. The delivery rate is just under the maximal rate at which rats can drink from a spout and accurately equates to actual intake. Rats were placed into the drinking cages in quick succession, facing away from the spouts, and drinking sessions started in all cages simultaneously by remote control, as the experimenter left the cubicle. One data record for mepyramine, two data records for metergoline, two data records for cyproheptadine and one data record for SB 242084 with mepyramine were lost due to technical difficulties.

2.4. Analysis

One-way analyses of variance (ANOVA) with repeated measures on drug conditions, followed by post hoc Dunnett's t tests, was used to compare treatment means to vehicle, in respect of total intake and latency to first lick and interlick intervals in the mepyramine, metergoline and cyproheptadine experiments. A two-way ANOVA (SB 242084 × Mepyramine) with repeated measures on drug conditions, followed by post hoc Dunnett's t tests, was used to analyse total intake and latency to first lick and interlick intervals in the SB 242084 with mepyramine experiment. In addition, the data were further analysed by examining the numbers of clusters (runs of licks separated by interlick intervals of more than 500 ms) and mean cluster size (Davis, 1973; Davis and Smith, 1992; Spector et al., 1998).

Table 1
Parameters describing the behavioural microstructure of 10% intralipid drinking following administration of (a) mepyramine, (b) metergoline, (c) cyproheptadine or (d) SB 242084 with mepyramine

Compound and dose (mg/kg)	Latency to first lick ($\sqrt{}$s)	Interlick interval (1/100 s)	Number of clusters	Cluster size
a) Mepyramine				
Saline	4.29 ± 0.55	13.67 ± 0.14	39.33 ± 6.46	76.50 ± 8.05
1.0	5.63 ± 0.77	14.00 ± 0.13	39.00 ± 6.02	84.27 ± 11.37
3.0	5.03 ± 0.81	14.00 ± 0.21	36.92 ± 5.19	83.08 ± 10.29
10.0	3.63 ± 0.66	15.00 ± 0.12**	32.67 ± 4.14	52.00 ± 5.60*
b) Metergoline				
Vehicle	4.94 ± 0.67	14.00 ± 0.33	39.75 ± 6.32	63.33 ± 7.23
0.3	3.69 ± 0.61	14.67 ± 0.40	46.50 ± 5.41	47.92 ± 4.78**
1.0	2.61 ± 0.73	15.09 ± 0.34**	41.82 ± 4.28	44.09 ± 6.10**
3.0	3.73 ± 1.05	16.36 ± 0.49**	52.09 ± 6.79*	32.64 ± 3.92**
c) Cyproheptadine				
Vehicle	2.97 ± 0.50	13.45 ± 0.21	55.36 ± 6.12	60.73 ± 9.74
0.1	1.73 ± 0.42	14.25 ± 0.18**	55.58 ± 5.52	70.83 ± 10.79
0.3	2.67 ± 0.51	14.42 ± 0.23**	67.25 ± 7.88	63.83 ± 11.57
1.0	2.33 ± 0.49	14.73 ± 0.30**	83.82 ± 8.68*	40.09 ± 5.04
d) 3 mg/kg SB 242084 + 1 mg/kg mepyramine				
Veh + sal	2.23 ± 0.66	14.17 ± 0.11	73.50 ± 13.11	59.62 ± 12.63
Veh + mep	2.51 ± 0.62	14.58 ± 0.15	64.83 ± 11.34	54.50 ± 9.84
SB + sal	1.46 ± 0.47	14.42 ± 0.15	64.33 ± 8.82	43.25 ± 7.05
SB + mep	2.46 ± 0.72	14.45 ± 0.16	61.09 ± 9.29	49.64 ± 11.83

Mean latency to first lick is shown as square root ($\sqrt{}$) of seconds; means of individual median interlick intervals are shown in 1/100 of seconds; cluster number indicates the mean number of clusters of licking and cluster size indicates the mean number of licks per cluster.

* Significant difference ($P < .05$; Dunnett's t test) from vehicle.

** Significant difference ($P < .01$; Dunnett's t test) from vehicle.

Data for latency to the first lick was transformed prior to analysis using a square root transformation to minimise positive skew (Howell, 1997). Interlick intervals (defined as every interval between the first to the last lick of the drinking session) were extracted from the data as median scores to decrease the impact of extreme scores present towards the end of the session (Howell, 1997). Thus, the interlick interval results presented in Table 1 represent the group means of the individual median scores.

3. Results

3.1. Mepyramine

Mepyramine reduced intralipid intake [$F(3,32)=21.59$, $P<.001$], and is significant at 10 mg/kg ($P<.01$), as seen in the intake graph of Fig. 1a. Table 1a shows the microstructural analysis for mepyramine and ANOVA revealed no significant effect on latency. Mepyramine increased median interlick interval [$F(3,32)=34.27$, $P<.001$] but did not affect the number of clusters of licking and reduced mean cluster size [$F(3,32)=6.09$, $P=.002$].

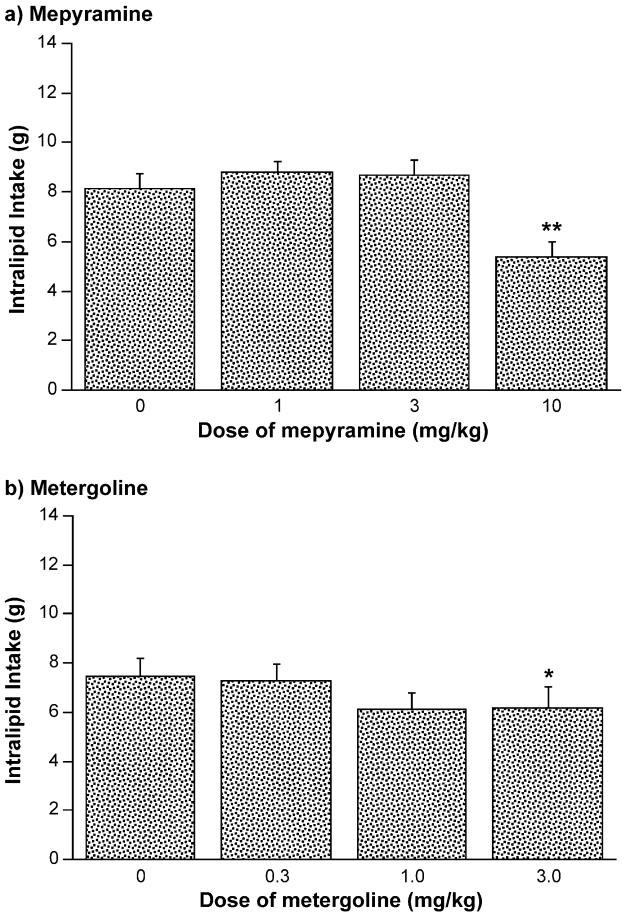

Fig. 1. Mean 10% intralipid intake (in grams) following administration of (a) mepyramine or (b) metergoline. Vertical lines show standard errors. * Significant difference ($P<.05$; Dunnett's t test) from vehicle; ** significant difference ($P<.01$; Dunnett's t test) from vehicle.

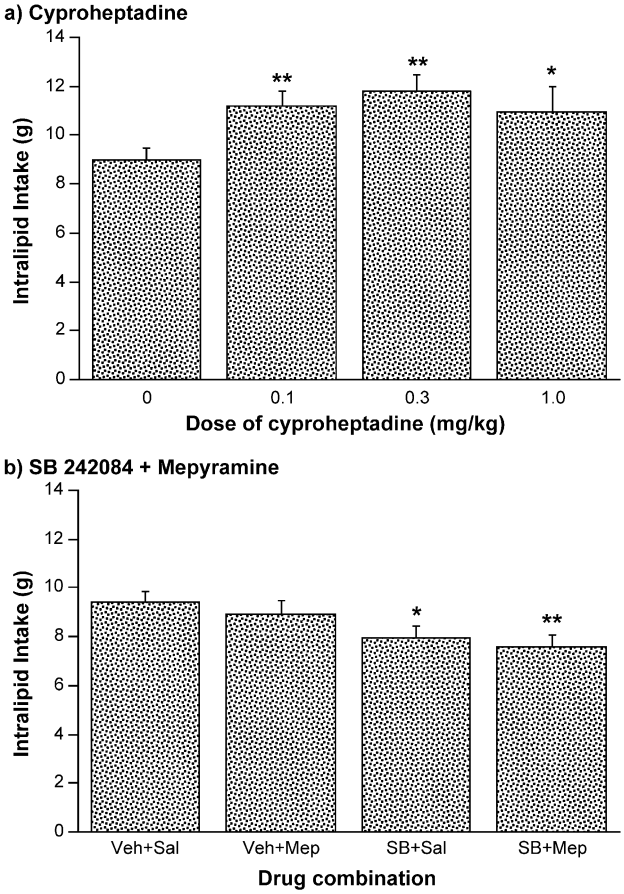

Fig. 2. Mean 10% intralipid intake (in grams) following administration of (a) cyproheptadine or (b) SB 242084 (3 mg/kg) with mepyramine (1.0 mg/kg). Vertical lines show standard errors. * Significant difference ($P<.05$; Dunnett's t test) from vehicle; ** significant difference ($P<.01$; Dunnett's t test) from vehicle.

3.2. Metergoline

Metergoline reduced intralipid intake [$F(3,31)=3.42$, $P=.029$; Fig. 1b], and is significant at 3.0 mg/kg ($P<.05$). The microstructural analysis for metergoline is shown in Table 1b; metergoline increased interlick intervals [$F(3,31)=26.17$, $P<.001$]. The number of clusters of licking was increased [$F(3,31)=3.20$, $P=.037$] and mean cluster size was reduced [$F(3,31)=13.34$, $P<.01$] by metergoline.

3.3. Cyproheptadine

Cyproheptadine produced a significant increase in intralipid intake [$F(3,31)=6.15$, $P=.002$; Fig. 2(a)] of 2.8 g (31%, $P<.01$) at the maximally effective dose of 0.3 mg/kg. The microstructural analysis for cyproheptadine is shown in Table 1c and ANOVA revealed no significant effect on latency. The median interlick interval

was significantly increased by cyproheptadine [$F(3,31) = 13.27$, $P < .001$]. The number of clusters of licking was significantly increased by cyproheptadine [$F(3,31) = 3.99$, $P = .016$].

3.4. SB 242084 with mepyramine

A two-way ANOVA on intralipid intake revealed a significant reduction by SB 242084 [$F(1,11) = 14.48$, $P = .003$] with no main effect of mepyramine and no (SB 242084 × Mepyramine) interaction, as shown in Fig. 2b. Table 1d shows the behavioural microstructure data. There were no main effects of SB 242084 or mepyramine and there were no (SB 242084 × Mepyramine) interactions, in respect of latency, interlick interval, number of clusters or mean cluster size.

4. Discussion

It has been suggested that the antiserotonergic and/or antihistaminergic properties of some antipsychotic agents may be the neurochemical mechanism that is wholly, or partly, responsible for the weight gain that is associated with their use (Stanton, 1995; Baptista, 1999; Casey and Zorn, 2001). The intralipid-drinking paradigm reported here has previously revealed hyperphagia in rats treated with clozapine, olanzapine or quetiapine (Hartfield et al., 2001, 2002, 2003). The present data provide additional insights into the possible contributions of serotonergic and histaminergic systems to the modulation of intralipid consumption by rats.

4.1. Intake

Neither metergoline (5-$HT_{1/2}$ antagonist) nor SB 242084 (5-HT_{2C} antagonist) produced increased intralipid intake. Furthermore, mepyramine (H_1 antagonist) failed to increase intralipid intake, either when administered alone or when administered in combination with SB 242084. In marked contrast, cyproheptadine (mixed H_1, 5-$HT_{2A/2B/2C}$ and muscarinic antagonist) produced substantial intralipid hyperphagia.

4.2. Ingestive behaviour

The behavioural data reveal several significant points. None of the drug treatments led to increases in the latency to the first lick, suggesting that there were no substantial sedative effects that might have potentially reduced responding at the beginning of the test session. Metergoline, mepyramine and cyproheptadine all produced dose-related increases in interlick intervals, demonstrating that, in each case, as the dose was increased, licking was slowed. Metergoline and cyproheptadine both led to dose-related increases in cluster number. The number of clusters of licking during a drinking session can be taken as a measure of the post-ingestive negative feedback signal that inhibits ingestive behaviour as the session progresses (Davis and Levine, 1977; Davis and Smith, 1988, 1992; Davis et al., 2000). Therefore, in the present study, metergoline and cyproheptadine treatment may be leading to a delay in the post-ingestive satiety signal. Mepyramine and metergoline treatment resulted in dose-related decreases in cluster size. Davis and Smith (1992) have argued that reductions in cluster size are associated with decreased palatability of the test solution. One interpretation of this effect of mepyramine and metergoline would, therefore, be in terms of a reduction in incentive value or palatability of the test solution.

The pattern of intralipid hyperphagia, accompanied by increased number of clusters and neutral cluster size following cyproheptadine treatment, is the same as that previously reported for olanzapine (Hartfield et al., 2001, 2003). Thus, regardless of the particular behavioural interpretation of the drug-induced effects of these two compounds, they are likely to have arisen through a similar mechanism. Cyproheptadine has been previously reported to substitute for clozapine in drug discrimination studies (Hoenicke et al., 1992; Kelley and Porter, 1997).

4.3. Serotonergic mechanisms

Considering the extensive evidence that 5-HT has an inhibitory effect on food intake, it could seem unexpected that metergoline, a 5-$HT_{1/2}$ antagonist, failed to increase intralipid consumption. Previously, metergoline has been reported to attenuate the reductions in feeding observed, following central 5-HT administration (Currie and Coscina, 1996). However, metergoline, when administered alone, only appears to induce hyperphagia in specific situations, such as at the start of the dark part of the light–dark cycle (Currie and Coscina, 1996) or immediately following a prefeeding session (Fletcher, 1988), and may also preferentially affect carbohydrate intake (Stallone and Nicolaidis, 1989). Therefore, failure to demonstrate hyperphagia in the present paradigm, utilising fat drinking in the light part of the photoperiod, does not contradict either the 5-HT or metergoline literature. Furthermore, our data suggests that 5-$HT_{1/2}$ receptor blockade is not sufficient to produce intralipid hyperphagia, although a full dose–response study is required to further validate this conclusion.

Similarly, SB 242084 (5-HT_{2C} antagonist) administered alone failed to increase intralipid intake. SB 242084 has been shown to attenuate 5-HT_{2C} receptor agonist-induced reductions in feeding, but it has not been shown to produce hyperphagia when administered alone (Kennett et al., 1997; Clifton et al., 2000; Vickers et al., 2001). In addition, olanzapine has been found to act as an inverse agonist, rather than an antagonist, at 5-HT_{2C} receptors (Herrick-Davis et al., 2000), and more revealing results may be obtained using SB 243213, a recently characterised 5-HT_{2C} receptor inverse agonist (Bromidge et al., 2000). Nevertheless, our data suggest that 5-HT_{2C} receptor block-

ade alone may not be sufficient to produce intralipid hyperphagia.

4.4. Histaminergic mechanisms

There is an indication from human data that H_1 receptor affinity may be a more accurate predictor of weight gain following antipsychotic treatment in humans than 5-HT_{2C} receptor affinities (Wirshing et al., 1999; Kroeze et al., 2003), but mepyramine failed to increase intralipid intake in rats. Mepyramine attenuates the reduction of feeding caused by central H (Lecklin et al., 1998); however, it is not reported to lead to hyperphagia when administered alone. Indeed, long-term intake of mepyramine in rats is associated with reductions of body weight in rats (Greenman et al., 1995). Thus, mepyramine's failure to increase intralipid intake does not contradict either the H or mepyramine literature. Furthermore, it appears that simple H_1 receptor antagonism alone is not sufficient to produce intralipid hyperphagia.

4.5. Serotonergic and histaminergic interactions

It has been suggested that a combination of 5-HT and H_1 antagonism may be the mechanism responsible for antipsychotic-associated weight gain in humans (e.g., Baptista, 1999; Casey and Zorn, 2001). In rats, drinking intralipid cyproheptadine (mixed 5-$HT_{2A/2B/2C}$, H_1 and muscarinic antagonist) produced a marked hyperphagia. Cyproheptadine has previously been reported to increase food intake in rats (Orthen-Gambill, 1988) and produce weight gain in humans (Silverstone and Schuyler, 1975). Thus, the 5-$HT_{2A/2B/2C}$, H_1 and muscarinic receptor blockade provided by cyproheptadine are sufficient to produce intralipid hyperphagia. The simple combination of 5-HT_{2C} and H_1 receptor blockade does not, however, appear to be sufficient to produce intralipid hyperphagia, since SB 242084 (5-HT_{2C} antagonist) coadministered with mepyramine (H_1 antagonist) reduced intake. But as SB 242084 at 3 mg/kg alone reduced intralipid intake, it is possible that a lower dose, in combination with mepyramine, would have resulted in elevated intake.

4.6. General discussion

There are several possible explanations for the ability of cyproheptadine to enhance intralipid intake, despite an absence of effect with treatments intended to replicate part of it's neurochemical profile. Further investigation of the relevant pharmacokinetic variables actions (e.g., maximum receptor occupancy, drug doses, route and time of administration) may be required before coadministration of selective compounds can mimic a drug with multiple actions.

A second possibility is that 5-HT_{2C} and/or H_1 antagonism are not critical to antipsychotic-associated weight gain. For example, both olanzapine and cyproheptadine have affinity for muscarinic receptors, whereas the combination of SB 242084 and mepyramine would not be expected to produce appreciable muscarinic blockade. However, there is no evidence that we are aware of, suggesting muscarinic antagonists increase feeding in either rats or humans.

It is also possible that 5-HT_{2A} receptors are involved in the hyperphagic response for intralipid. Indeed, clozapine, olanzapine and cyproheptadine all have high affinity for 5-HT_{2A} receptors and act as antagonists. 5-HT_{2A} receptors have been shown to play a role in feeding, specifically that activation of 5-HT_{2A} receptors inhibits the hyperphagia induced by neuropeptide Y (NPY) (Samanin and Garattini, 1996; Currie and Coscina, 1998). 5-HT_{2A} receptor antagonists can reverse the 5-HT_{2A} receptor agonist attenuation of NPY-induced feeding (Currie et al., 1999). In addition, stimulation of 5-HT_{2A} receptors reduces food intake and disrupts the continuity of feeding in rats (Simansky, 1996). Therefore, by producing blockade of 5-HT_{2A} receptors, clozapine, olanzapine and cyproheptadine may be preventing NPY regulation during feeding and enhancing the continuation of feeding. However, the atypical antipsychotic ziprasidone has high affinity for 5-HT_{2A} receptors (Schotte et al., 1996) and, yet, ziprasidone treatment is not associated with weight gain in humans (e.g., Allison et al., 1999) or intralipid hyperphagia in rats (Hartfield et al., 2002). It is possible that the combination of 5-HT_{2A}, 5-HT_{2C} and H_1 receptor blockade accounts for clozapine-, olanzapine- and cyproheptadine-associated intralipid hyperphagia; however, ziprasidone also demonstrates similar binding affinities at these receptors (Schotte et al., 1996). In addition, quetiapine is associated with weight gain in humans (e.g., Allison et al., 1999) and intralipid hyperphagia in rats (Hartfield et al., 2002), but does not have high affinity at either 5-HT_{2A} or 5-HT_{2C} receptors (Bymaster et al., 1996a). As discussed earlier, in addition to serotonergic and histaminergic activity, cyproheptadine possesses a moderate affinity for muscarinic receptors that may contribute to the overall activity profile of this agent (Ketelaars and Bruinvels, 1989; Richards, 1991).

Atypical antipsychotics like olanzapine and clozapine have broad pharmacological profiles that encompass several receptor subtypes of numerous neurochemical systems and many of these receptors and systems have been found to affect feeding behaviour and weight regulation. Casey and Zorn (2001) consider the binding profiles of antipsychotic drugs and suggest that the relevant combinations may include adrenergic α_1 receptors with 5-HT_{2C} and H_1, or just H_1 and α_1. Clozapine and olanzapine have high in vitro affinity for α_1 and H_1 receptors. However, in vivo α_1 receptor antagonism has only been demonstrated for olanzapine at doses above 10 mg/kg ip (Bymaster et al., 1996b). This is much higher than the doses (0.3–1 mg/kg ip) at which intralipid hyperphagia has been demonstrated (Hartfield et al., 2001, 2003) and well into a dose range, over which intake is inhibited (>3 mg/kg/ip). Baptista et al. (2002) recently argued that compounds exhibiting high

affinity for muscarinic H_1 and 5-HT_{2C} receptors, combined with low affinity for D_2 and 5-HT_{1A} receptors, such as clozapine, were associated with greater weight gain. Conversely, antipsychotic agents, such as ziprasidone, which possess high D_2 and 5-HT_{1A} receptor affinity with low H_1 and muscarinic receptor affinity, are not associated with weight gain (Baptista et al., 2002).

Potent dopamine receptor antagonism possibly causes or contributes to weight gain associated with typical antipsychotic agents such as haloperidol. Dopamine blockade, particularly at D2 receptors in tuberoinfundibular neurones releases the pituitary from inhibitory control, resulting in elevated secretion of the pituitary hormone prolactin, which ordinarily controls lactation. Hyperprolactinaemia has been associated with antipsychotic use for many years, but the exact mechanism of how elevated prolactin leads to weight gain is not clear (Baptista, 1999). Nevertheless, there is a positive correlation between serum prolactin levels and weight gain in humans (Wang et al., 1987) and there is some suggestion that the hyperprolactinaemia association with weight gain may be related to gonadal steroids.

For example, during chronic administration in female rats, the antipsychotic compound sulpiride causes hyperprolactinaemia and changes in gonadal steroids, particularly increased androgen levels (Baptista et al., 1997). This is thought to be responsible for the observation of bodyweight gain and increased food intake (Baptista et al., 1997). Increased androgen levels in female rats is associated with increased appetite and fat deposition (Wade and Gray, 1979; Wade and Schneider, 1992). Restricted food intake prevents sulpiride-induced weight gain in this model, but there is still a suggestion of hyperprolactinaemia (Baptista et al., 1998).

Hyperprolactinaemia induced by potent dopamine receptor blockade is unlikely to explain atypical antipsychotic-associated weight gain because although clozapine and olanzapine have been shown to increase prolactin, these elevations are only transient and levels return to normal within a few hours (Markianos et al., 1999; Esel et al., 2001; Turrone et al., 2002).

There are several other mechanisms that may be wholly or partly responsible for antipsychotic-associated weight gain. Baptista (1999) argues that antipsychotic compounds could be altering appetite regulation, or having metabolism or endocrine effects and that any of these may lead to weight gain. Sedation, leading to reduced activity, might also promote weight gain in antipsychotic-treated patients (Stanton, 1995). Casey and Zorn (2001) point out a complete understanding of the neurochemical, peptide and hormonal control of food intake. Weight control is still yet to be achieved and, accordingly, the full interaction of antipsychotic drugs with these systems has yet to be investigated.

In conclusion, these experiments have established several key points. Specifically, increased intralipid intake in rats is not induced by simple H_1, 5-$HT_{1/2}$ or 5-HT_{2C} receptor blockade alone or by a combination of 5-HT_{2C} and H_1 receptor blockade. However, cyproheptadine did increase intralipid intake, suggesting that simultaneous antagonism of 5-$HT_{2A/2B/2C}$ and H_1 receptors, plus some muscarinic antagonism, may contribute to the intralipid hyperphagia observed in the present studies. A similar mechanism may underlie the hyperphagic response to intralipid observed in rats following treatment with either clozapine or olanzapine.

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