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Pharmacology, Biochemistry and Behavior 73 (2002) 545–550

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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mCPP-induced hypophagia in rats is unaffected by the profile of dietary unsaturated fatty acids

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Received 11 February 2002; received in revised form 11 February 2002; accepted 29 March 2002

Abstract

The n-3 and n-6 polyunsaturated fatty acids (PUFAs) have been shown to modify central serotonergic parameters relevant to ingestive behavior. Evidence suggests an association between the 5-HT_{2C} receptor and fat intake. The present research sought to examine the role of the 5-HT_{2C} receptor subtype on food intake when diets with different fatty acid compositions are consumed. The effects of 1-(3-chlorophenyl)piperazine (mCPP) on consumption of both low-fat (Experiment 1) and high-fat diets (Experiment 2) differing in their predominant PUFA profiles were compared in rats. Regardless of the PUFA profile, mCPP induced hypophagia within each experiment. Although the present results lend further support to a large body of evidence demonstrating the ability of mCPP to reduce food intake, they do not support the idea that the essential fatty acid composition of the diet can differentially modulate mCPP-induced hypophagia.

Keywords: Agonist; Essential fatty acid; Fat; 5-HT_{2C}; Flaxseed; Food intake; Ingestive behavior; Linoleic acid; Linolenic acid; Safflower; Serotonin

1. Introduction

Serotonin (5-HT) is one of several neuromodulators thought to be involved in attenuating food intake (Simansky, 1996). Originally, the ability of 5-HT to decrease food intake was believed to be carbohydrate specific (Wurtman and Wurtman, 1979); however, more recent evidence suggests that this may not be the case (Smith et al., 1998, 1999). In fact, pharmacological evidence has indicated that 5-HT may also be involved in the satiating effects of fat (Blundell et al., 1995).

Rats maintained on three-choice macronutrient diets reduce fat intake in response to increases in serotonergic activity via peripheral administration of 5-HT (Kanarek and Dushkin, 1988), the specific 5-HT reuptake inhibitor fluoxetine (Heisler et al., 1997, 1999; Weiss et al., 1991), and the mixed 5-HT agonist/reuptake inhibitors fenfluramine (Orthen-Gambill and Kanarek, 1982; Shor-Posner et al., 1986) or dexfenfluramine (Smith et al., 1998, 1999). Dexfenfluramine-induced reduction of fat intake is of particular interest given recent evidence that the hypophagia induced by dexfenfluramine is dependent upon activation of the 5-HT_{2C} receptor (Clifton et al., 2000; Vickers et al., 1999). Together, these studies suggest an association between the 5-HT_{2C} receptor and fat intake.

The discovery and characterization of multiple 5-HT receptor subtypes has made available new pharmacological tools for exploring the role of 5-HT in the control of feeding. The present investigation used the mixed 5-$HT_{2C/1B}$ receptor agonist 1-(3-chlorophenyl)piperazine (mCPP). Although mCPP is a mixed 5-$HT_{2C/1B}$ agonist, its affinity is greatest for the 2C receptor (Middlemiss and Tricklebank, 1992). Furthermore, the effects of mCPP on food intake are believed to be mediated by the 5-HT_{2C} receptor (Kennett and Curzon, 1991). While there are reports that mCPP induces hypophagia in rodents (Clifton et al., 1993; Dryden et al., 1996; Heslop and Curzon, 1999; Kennett and Curzon, 1988, 1991; Kennett et al., 1987; Kitchener and Dourish, 1994; Samanin et al., 1979; Simansky and Vaidya, 1990), it is not known if the fatty acid profile of the diet affects mCPP-induced hypophagia.

Fats are composed of fatty acids, which differ in several attributes including chain length, number of double bonds

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0091-3057/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0091-3057(02)00839-0

(degree of unsaturation), and location of the double bonds. Fatty acids with more than one double bond are called polyunsaturated fatty acids (PUFAs). n-3 fatty acids are PUFAs with the first double bond between the third and fourth carbons in the chain. n-6 fatty acids are also PUFA, but the first double bond is located between the sixth and seventh carbon. Essential fatty acids are 18-carbon PUFAs that must be supplied by the diet. These are linoleic acid (18 carbons and 2 double bonds [18:2; n-6]), and linolenic acid (18 carbons and 3 double bonds [18:3; n-3]).

n-3 and n-6 PUFAs have been reported to differentially influence ingestive behavior in some studies (Greenberg, 1998; Tsuruta et al., 1999), and also have been reported to modify central serotonergic parameters with potential relevance to ingestive behavior (Chalon et al., 1998). Whether dietary fatty acids can differentially influence ingestive behavior via the 5-HT_{2C} receptor, however, has not been investigated.

Thus, the present research sought to examine the role of the 5-HT_{2C} receptor subtype on food intake regulation when diets with different fatty acid compositions are consumed. While controlling for potential confounds of the diet such as acceptability, nutrient composition and energy density, the present research sought to determine if energy intake of diets mixed with oils rich in one of the essential fatty acids (either linoleic [18:2; n-6; provided by safflower oil] or linolenic [18:3; n-3; provided by flaxseed oil] acid) is differentially sensitive to mCPP-induced hypophagia. Based on recent evidence that diets rich in n-3 fatty acids can induce lower regional MAO activity and higher 5-HT levels in the brain compared to diets rich in n-6 fatty acids (Chalon et al., 1998), we speculated that rats fed a diet high in linolenic (n-3) acid would be less sensitive to mCPP-induced hypophagia than rats fed a diet high in linoleic (n-6) acid, due to possible receptor down-regulation and/or decreased sensitivity. If correct, mCPP would induce hypophagia in all rats, but higher dosages would be required in the flaxseed oil group to produce an effect.

2. Methods and procedures

The Pennsylvania State University Institutional Animal Care and Use Committee approved all procedures. Two experiments of similar experimental design were conducted. In the first experiment, the effects of mCPP on consumption of low-fat diets differing in their predominant PUFA profiles are compared. In the second experiment, the effects of mCPP on consumption of high-fat diets differing in their predominant PUFA profiles are compared.

2.1. Subjects

Twenty-eight male Sprague–Dawley (Harlan, Indianapolis, IN) rats (age=4 months) were used in each of the two experiments (total n=56 rats). Animals were individually housed in hanging cages in a temperature- and humidity-controlled facility, with a 12:12 light cycle.

2.2. Diets

For 34 days prior to the initiation of the present investigation, rats in Experiment 1 were maintained on either a low-fat safflower (The Hain Food Group; Uniondale, NY) or a low-fat flaxseed (generously donated by Spectrum Naturals; Petaluma, CA) oil diet (% fat [wt/wt]=9.28; energy density=3.585 kcal/g). In Experiment 2, rats were maintained for 34 days on either a high-fat safflower oil or a high-fat flaxseed oil diet (% fat [wt/wt]=23.60; energy density=4.44 kcal/g). All diets were formulated by mixing either safflower or flaxseed oil with a constant formula rodent meal (Laboratory Rodent Diet 5001, PMI Feeds, Richmond, IN; % macronutrients by energy [physiological fuel values]: 28.36% protein, 12.27% fat, and 59.38% carbohydrate; by weight: 23.40% protein, 4.50% fat [ether extract] and 49.00% carbohydrate [nitrogen-free extract]; 3.3 kcal/g physiological fuel value). The choice of oils for the present investigation was based upon their dominant fatty acid composition. Safflower oil was chosen because of its high concentration of linoleic acid (18:2, n-6; 72.04%), while flaxseed oil was chosen because of its high concentration of linolenic acid (18:3, n-3; 59.40%). To reduce the likelihood of oxidation, *tert*-butyl-hydroquinone (TBHQ; Acros Organics/Fisher Scientific, Pittsburgh, PA) was added to the diet at the time of mixing in the amount of 0.02% (wt/wt) of the additional oil. Within each experiment, then, the diets were similar in energy density and macronutrient distribution. The macronutrient and essential fatty acid compositions of the diets used in Experiments 1 and 2 are summarized in Tables 1 and 2, respectively.

2.3. Drug

mCPP (MW=270; Tocris Cookson; Ballwin, MO), a 5-$HT_{2C/1B}$ receptor agonist, was tested at the following four dosages in both experiments: 0 (vehicle), 0.3, 1.0 and 3.0 mg/kg. mCPP was mixed with deionized distilled water and administered intraperitoneally at a volume of 1 ml/kg. Dosages were chosen based on previous literature

Table 1
Low-fat oil diet composition[a]

	% Weight		% Energy	
	Safflower	Flaxseed	Safflower	Flaxseed
Protein	22.23	22.23	24.80	24.80
Carbohydrate	46.55	46.55	51.92	51.92
Fat[b]	9.28	9.28	23.28	23.28
Linoleic acid (18:2)	4.76	1.91	10.20	0.10
Linolenic acid (18:3)	0.08	3.04	3.03	7.50

[a] Energy density=3.585 kcal/g.
[b] Includes fat from the constant-formula rodent meal and the added oil.

Table 2
High-fat oil diet composition[a]

	% Weight		% Energy	
	Safflower	Flaxseed	Safflower	Flaxseed
Protein	18.72	18.72	16.86	16.86
Carbohydrate	39.20	39.20	35.31	35.31
Fat[b]	23.60	23.60	47.83	47.83
Linoleic acid (18:2)	15.57	4.15	32.62	7.64
Linolenic acid (18:3)	0.12	11.95	0.19	25.71

[a] Energy density=4.44 kcal/g.
[b] Includes fat from the constant-formula rodent meal and the added oil.

in which mCPP was reported to induce hypophagia (Kitchener and Dourish, 1994), but not hypolocomotion (Bonhaus et al., 1997) or hypoactivity (Kitchener and Dourish, 1994).

2.4. Procedure

Two hours prior to lights out, the food was removed from the cages. One hour into the dark cycle (3 h following the removal of the food) each rat was removed from his cage, weighed, injected intraperitoneally with his assigned dosage, and returned to his cage. One-half hour postinjection (similar to previously reported pretreatment times (Kennett and Curzon, 1988, 1991; Kitchener and Dourish, 1994; Samanin et al., 1979)), the rat's bowl of food was returned to his cage. A similar feeding protocol, which incorporates mild food deprivation near the beginning of the dark cycle, has been used successfully by other investigators to stimulate food intake in order to demonstrate the attenuation by anorectic agents (Heisler et al., 1997, 1999; Shor-Posner et al., 1986; Weiss et al., 1991). Food intake was measured at 1, 2 and 20 h postinjection.

All rats received all dosages (dosing sequences randomly assigned) during the course of both investigations, with 96 h elapsing between each of the administered dosages. Prior to each injection, each rat's 24-h food intake had returned to within 10% of his mean 24-h baseline intake. Mean 24-h baseline intake was based on three consecutive 24-h food intake measurements obtained immediately prior to the initiation of the present experiments.

2.5. Statistical analysis

All analyses were conducted utilizing either SAS for Windows (Version 7.00; SAS Institute, Cary, NC) or GraphPad Prism (Version 3.0; GraphPad Software, San Diego, CA). When missing data were encountered, group means were calculated and inserted in place of the missing values (Tabachnick and Fidell, 1996). The Kolmogorov–Smirnov test was used to assess all data for deviations from Gaussian distribution (normality). For both experiments, all data were found to be normally distributed.

Intake data were analyzed both cumulatively and non-cumulatively. That is, food intake data were analyzed at 0–1, 0–2, 0–20, 1–2 and 2–20 h. The data were analyzed by two-way (Dosage×Group) analysis of variance (ANOVA), with dosage as the repeating measure and group as the between-groups factor. In the absence of a significant interaction, main effects were evaluated. When significant, main effects were followed by multiple comparisons procedures using Bonferroni *t* tests.

The body weight data were analyzed by two-way (Group×Time) ANOVA, with time as the repeating measure and group as the between groups factor. In the absence of a significant interaction, main effects were evaluated.

3. Results

3.1. Energy intake

Energy (see Figs. 1 and 2 and Tables 3 and 4) intake was attenuated similarly in both experiments. During the first hour, mCPP induced a similar dosage-dependent decrease in energy intake when both low-fat (Experiment 1, Fig. 1A) and high-fat (Experiment 2, Fig. 2A) diets were consumed [Experiment 1: two-way (Dosage×Group) ANOVA $F(3,78)=0.73$, NS; main effect (dosage) ANOVA $F(3,78)=130.13$, $P<.0001$, $P<.05$ Bonferroni *t* tests; Experiment 2: two-way (Dosage×Group) ANOVA $F(3,78)=0.47$, NS; main effect (dosage) ANOVA $F(3,78)=66.90$, $P<.0001$, $P<.05$ Bonferroni *t* tests].

In Experiment 1, less energy was consumed during the first hour by the low-fat safflower oil group than the low-fat flaxseed oil group at all dosages, including the vehicle [Experiment 1: main effect (group) ANOVA $F(1,26)=13.38$, $P<.005$, $P<.05$ Bonferroni *t* tests]. While this result might suggest a differential effect of dietary oils on food intake, similar effects were not detected in Experiment 2, nor was such an effect found at any other time point [Experiment 2: main effect (group) ANOVA $F(1,26)=0.01$, NS] (see Figs. 1 and 2 and Tables 3 and 4). This result was not due to differences in preinjection energy intake. That is, there were no differences in 24-h preinjection energy intakes between the low-fat safflower oil group and the low-fat flaxseed oil group [two-way (Dosage×Group) ANOVA $F(3,78)=0.74$, NS; main effect (group) ANOVA $F(1,26)=0.01$, NS; main effect (dosage) ANOVA $F(3,78)=0.44$,NS].

In both experiments, cumulative energy intake was still attenuated by mCPP at 2 and 20 h postinjection [Figs. 1B, C and 2B, C; cumulative 2-h energy intake: Experiment 1: main effect (dosage) ANOVA $F(3,78)=54.74$, $P<.0001$, $P<.05$ Bonferroni *t* tests; Experiment 2: main effect (dosage) ANOVA $F(3,78)=57.62$, $P<.0001$, $P<.05$ Bonferroni *t* tests; cumulative 20-h energy intake: Experiment 1: main effect (dosage) ANOVA $F(3,78)=8.92$, $P<.0001$, $P<.05$ Bonferroni *t* tests; Experiment 2: main effect (dosage) ANOVA $F(3,78)=14.73$, $P<.0001$, $P<.05$ Bonferroni *t* tests].

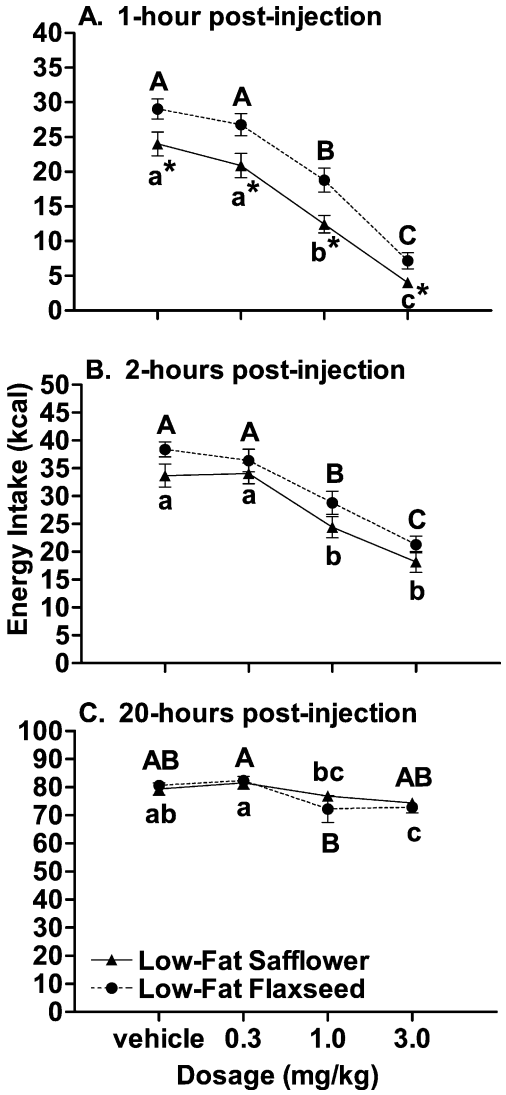

Fig. 1. The effect of mCPP on cumulative energy intake (mean ±S.E.M.) at 1 h (A), 2 h (B) and 20 h (C) postinjection in rats maintained on low-fat oil diets. If no error bar is visible, then it is included in the symbol. Different lowercase letters indicate significant differences between dosages within the safflower oil diet group ($P<.05$). Different uppercase letters indicate significant differences between dosages within the flaxseed oil diet group ($P<.05$). *Indicates at a specific dosage that energy intake of the safflower oil diet was significantly less than energy intake of the flaxseed oil diet ($P<.05$).

These cumulative reductions, however, were probably due to effects during the first hour postinjection, since energy intake during the second hour (hour 1–2) was not reduced by mCPP in either experiment [Experiment 1: main effect (dosage) ANOVA $F(3,78)=0.0037$, $P<.005$; one-way (dosage for safflower oil group) ANOVA $F(3,13)=2.11$, NS; one-way (dosage for flaxseed oil group) ANOVA $F(3,13)=3.93$, $P<.05$, $P<.05$ Bonferroni t tests showing an increase in food intake; Experiment 2: main effect (dosage) ANOVA $F(3,78)=0.24$, $P<.05$; one-way (dosage for safflower oil group) ANOVA $F(3,13)=1.25$, NS; one-way (dosage for flaxseed oil group) ANOVA $F(3,13)=2.60$, NS].

Furthermore, from hour 2 to hour 20 after injection of mCPP, energy intake was increased in both experiments, probably due to rebound feeding [Experiment 1: main effect (dosage) ANOVA $F(3,78)=11.55$, $P<.0001$; one-way (dosage for safflower oil group) ANOVA $F(3,39)=13.26$, $P<.0001$, $P<.05$ Bonferroni t tests; one-way (dosage for flaxseed oil group) ANOVA $F(3,13)=3.96$, $P<.05$, $P<.05$ Bonferroni t tests; Experiment 2: main effect (dosage) ANOVA $F(3,78)=15.93$, $P<.0001$; one-way (dosage for safflower oil group) ANOVA $F(3,39)=8.20$, $P<.005$, $P<.05$ Bonferroni t tests; one-way (dosage for flaxseed oil group) ANOVA $F(3,13)=9.29$, $P<.0001$, $P<.05$ Bonferroni t tests].

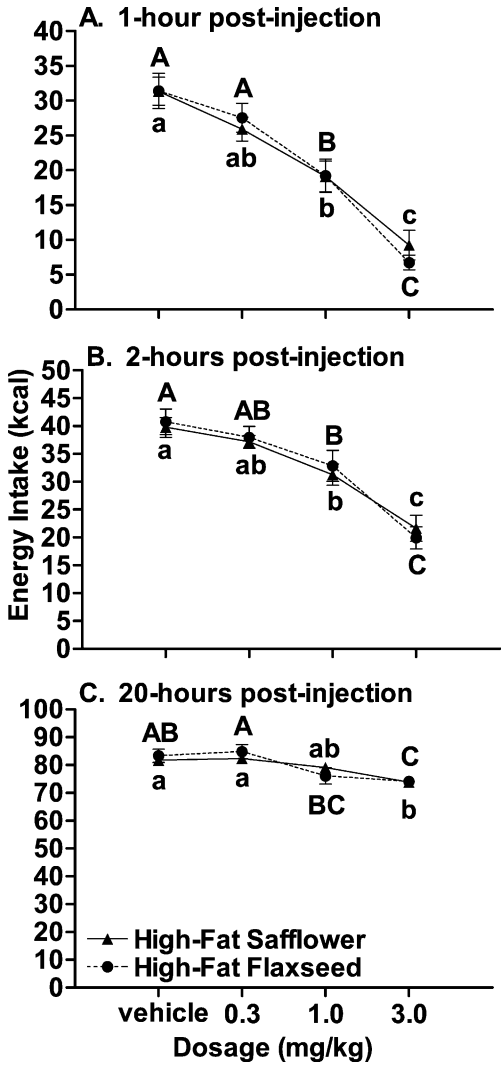

Fig. 2. The effect of mCPP on cumulative energy intake (mean ±S.E.M.) at 1 h (A), 2 h (B) and 20 h (C) postinjection in rats maintained on high-fat oil diets. If no error bar is visible, then it is included in the symbol. Different lowercase letters indicate significant differences between dosages within the safflower oil diet group ($P<.05$). Different uppercase letters indicate significant differences between dosages within the flaxseed oil diet group ($P<.05$).

Table 3
Experiment 1: Effect of mCPP on energy (kcal) intake (mean ±S.E.M.) of the low-fat (3.585 kcal/g) oil diet groups

Dosage (mg/kg)	Time (h)	Safflower	Flaxseed
0	1–2	9.7 ±1.1	9.4 ±1.3^{A}
0.3	1–2	13.2 ±1.2	9.6 ±1.4^{A}
1.0	1–2	12.0 ±1.8	10.0 ±1.3^{AB}
3.0	1–2	14.2 ±1.4	14.1 ±1.4^{B}
0	2–20	45.7 ±1.5^{a}	42.2 ±1.5^{A}
0.3	2–20	47.5 ±1.4^{ab}	46.0 ±1.8^{AB}
1.0	2–20	52.4 ±1.5^{bc,*}	43.5 ±3.5^{AB}
3.0	2–20	56.2 ±1.3^{c,*}	51.6 ±1.3^{B}

Different lowercase letters indicate significant differences between dosages within the safflower oil diet group (P<.05).
Different uppercase letters indicate significant differences between dosages within the flaxseed oil diet group (P<.05).
* Indicates at a specific dosage, energy intake of the safflower oil diet was significantly greater than energy intake of the flaxseed oil diet (P<.05).

3.2. Body weight

In neither experiment, nor at any time during the testing of mCPP, was there a significant difference in body weight between the safflower oil group and the flaxseed oil group [Experiment 1: initial body weights (g ±S.E.M.): safflower = 439 ±7, flaxseed = 429 ±5; final body weights (g ±S.E.M.): safflower = 452 ±8, flaxseed = 444 ±5; two-way (Day×Group) ANOVA $F(3,78)=0.7186$, NS; main effect (group) ANOVA $F(1,26)=0.96$, NS; Experiment 2: initial body weights (g ± S.E.M.): safflower = 444 ±7, flaxseed = 424 ±9; final body weights (g ±S.E.M.): safflower=461 ±7, flaxseed=443 ±9;.two-way (Day×Group) ANOVA $F(3,78)=0.94$, NS; main effect (group) ANOVA $F(1,26)=2.96$, NS).

4. Discussion

In the present investigation, the effect of mCPP on energy intake of rats maintained on food mixed with oil rich in either linoleic (18:2; n-6; safflower oil) or linolenic (18:3; n-3; flaxseed oil) acid was compared. mCPP induced hypophagia within each experiment; however, the same dosages reduced food intake regardless of the profile of dietary n-3 and n-6 fatty acids. In addition, significant differences between the flaxseed and safflower oil groups were not consistently demonstrated. These results were unexpected, given the evidence that oils rich in n-3 and n-6 fatty acids can differentially modify central serotonin levels and MAO activity (Chalon et al., 1998). Despite such evidence, the present results do not support the hypothesis that the essential fatty acid composition of the diet can influence the effect of 5-HT_{2C} receptor stimulation on ingestive behavior.

Food intake did appear to be differentially affected by the fatty acid composition of the diet during the first hour postinjections in Experiment 1, with rats on the safflower oil (18:2; n-6) diet consuming significantly less food than rats on the flaxseed oil (18:3; n-3) diet. While this result could be interpreted to suggest a greater satiating potency of n-6 rich diets, other data from this study do not support such a conclusion. Specifically, consumption of the safflower oil diet was not significantly less than consumption of the flaxseed oil diet at any other time point. More importantly, similar differences in intake were not seen in Experiment 2, when higher fat diets were consumed. Clearly, further work is needed to clarify whether n-6 and n-3 fatty acids can have differential effects on food intake under real-feeding conditions.

The present results are in agreement with previous reports of short-term reductions in food intake resulting from mCPP administration to rodents (Clifton et al., 1993; Dryden et al., 1996; Heslop and Curzon, 1999; Kennett and Curzon 1988, 1991; Kennett et al., 1987; Kitchener and Dourish, 1994; Samanin et al., 1979; Simansky and Vaidya, 1990). The present results are not in agreement, however, with reports that include measurement of 24-h food intake (Clifton et al., 1993; Kennett et al., 1987). In contrast to both the Clifton et al. (1993) and Kennett et al. (1987) reports, the present investigation demonstrated a small, albeit significant, attenuation of food intake by mCPP at 20 h postinjection. Differences in experimental design may account for this discrepancy. For example, this laboratory and Clifton et al. demonstrated a 10% decrease in food intake with a similar dosage of mCPP, but it was statistically significant in the present investigation and not in that of Clifton et al. Compared to the present investigation that used a within-subjects design with 14 rats per group, Clifton et al. used a between-subjects design with 9–10 rats/group. Thus, the possibility exists that Clifton et al. did not have sufficient power to detect significance with this small decrease in food intake. On the other hand, significant effects of mCPP were detected at 2 and 4 h postinjection, most likely because of the robust short-term effects that mCPP has on food intake.

In the Kennett et al. (1987). report, a higher dosage of mCPP (5.0 mg/kg) than that which attenuated intake in the

Table 4
Experiment 2: Effect of mCPP on energy (kcal) intake (mean ±S.E.M.) of the high-fat (4.44 kcal/g) oil diet groups

Dosage (mg/kg)	Time (h)	Safflower	Flaxseed
0	1–2	8.4 ±1.3	9.4 ±1.5
0.3	1–2	11.3 ±1.8	10.4 ±1.0
1.0	1–2	12.2 ±1.7	13.6 ±1.2
3.0	1–2	12.4 ±1.3	13.2 ±1.6
0	2–20	42.0 ±1.4^{a}	42.6 ±1.3^{A}
0.3	2–20	45.2 ±1.3^{a}	46.9 ±3.2^{A}
1.0	2–20	47.8 ±2.0^{ab}	43.3 ±2.4^{A}
3.0	2–20	52.3 ±1.7^{bc}	54.2 ±1.7^{B}

Different lowercase letters indicate significant differences between dosages within the safflower oil diet group (P<.05).
Different uppercase letters indicate significant differences between dosages within the flaxseed oil diet group (P<.05).

present study (3.0 mg/kg) did not decrease food intake at 24 h postinjection. This discrepancy between results may be a function of the time during the diurnal cycle when mCPP was injected. In the Kennett et al. investigation, mCPP was injected 7 h into the light phase; whereas, in the present investigation, it was injected 1 h into the dark phase. This is important because, in rats, intake varies across the diurnal cycle with more food being consumed during the dark phase than during the light phase (Tempel et al., 1989). Thus, in the Kennett et al. report, at 4 h postinjection, the food intake difference between rats injected with vehicle and rats injected with mCPP was approximately 1 g. In contrast, at only 2 h postinjection, in the current study, there was a difference of approximately 5 g between rats injected with vehicle and rats injected with mCPP. Upon metabolism of mCPP, normal feeding could resume. While rats in the Kennett et al. report could easily compensate for their intake deficit of 1 g, rats in the present investigation needed to consume five times that amount in order to completely compensate for their intake deficits. Indeed, intakes in the present experiment increased from hours 1–2 and hours 2–20, but were not high enough to compensate for the deficits incurred during the first hour.

The present results lend further support to a large body of evidence demonstrating the ability of mCPP to reduce food intake, but do not support the idea that the essential fatty acid composition of the diet can differentially modulate mCPP-induced hypophagia.

Acknowledgments

The authors would like to express their appreciation to Rachel Leal for her energetic dedication to this investigation.

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