Comparison of Central and Peripheral Administration of C75 on Food Intake, Body Weight, and Conditioned Taste Aversion

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Mice respond to fatty acid synthase (FAS) inhibitors by profoundly reducing their food intake and body weight. Evidence indicates that the central nervous system (CNS) may be the critical site of action; however, a peripheral contribution cannot be ruled out. We compared doses of the FAS inhibitor C75 in the CNS (third ventricle [i3vt]) and periphery (intraperitoneal [IP]) to reduce food intake and body weight in rats. Centrally, the threshold dose was 3 μg, whereas a dose of 10 mg/kg was required peripherally. Such data argue for FAS activity in the CNS as a potent target for the actions of C75. To control for nonspecific effects of FAS inhibition, we compared C75 administration in two models of illness, conditioned taste aversion and need-induced sodium appetite. Our results suggest the anorexia produced by IP C75 is accompanied by visceral illness, whereas the anorexia produced by i3vt is not. In addition, we placed animals in an indirect calorimeter after an IP injection of C75. We found that consistent with behavioral measures of visceral illness, peripheral C75 reduced heat expenditure and resulted in animals losing less weight than fasted control animals, suggesting that peripherally administered C75 has aversive properties. Understanding the mechanisms by which FAS inhibition in the CNS reduces food intake could lead to specific targets for the manipulation of energy balance and the treatment of obesity. Diabetes 51:3196–3201, 2002

For the body to maintain energy homeostasis, caloric intake needs to equal fuel utilization. In principle, this matching of energy intake with energy expenditure could occur on a meal-to-meal basis, as posited by the glucostatic hypothesis and related depletion-repletion models (1–3). Alternatively, the matching could occur over longer intervals, with food intake adjusting to energy use over days or weeks as suggested by research on free-feeding humans and animals (4,5). Although acute fluctuations of cellular metabolism generally have not been thought to contribute greatly to regulation of long-term energy balance (6–8), the recent demonstration that drugs that inhibit the enzyme fatty acid synthase (FAS), such as C75 or cerulenin, potently reduce food intake and cause profound weight loss in mice (9,10) suggests otherwise. During the fed state, fuels enter the cell. When there is an excess of cellular fuel, lipogenesis occurs, resulting from an increase in malonyl-CoA. Malonyl-CoA is both an intermediate in de novo synthesis of fatty acids and an allosteric inhibitor of carnitine palmitoyltransferase I, the enzyme that regulates the rate at which long-chain fatty acyl CoAs enter the mitochondria, where they are oxidized (11).

Although these results are intriguing, consideration of the use of pharmacologic agents to inhibit FAS as a treatment strategy for weight loss requires that several basic questions be addressed. First, to generalize the anorexia induced by FAS inhibitors across species, we examined the effect of C75 in rats. Second, because C75 presumably affects metabolism in many tissues, it is important to begin to understand the site of C75 action. We therefore assessed the relative potencies of C75 injected centrally or peripherally to reduce food intake and body weight. Third, we replicated and extended previous data about the anorexic potency of peripheral C75 in animals maintained on a high-fat diet. In addition, we assessed whether the efficacy of centrally administered (third ventricle [i3vt]) C75 was different depending on the amount of dietary fat because fat influences the rate of de novo fatty acid synthesis in several tissues. Finally, because suppression of food intake can occur for many reasons, including visceral illness, we investigated the hypothesis that either central or peripheral C75-induced anorexia was secondary to malaise. To do so, we compared the effects of C75 to those of the prototypical toxin lithium chloride (LiCl) on two measures sensitive to the presence of visceral illness in the rat. LiCl is known to produce emesis and reports of nausea in humans (12). In rats, which are not capable of emesis, LiCl produces a range of other symptoms that can be used to determine the presence of visceral illness (e.g., [13]). In addition to suppressing the intake of calories, visceral illness produces a reduction of need-induced NaCl intake (14) and a decrease in total energy expenditure (10). Visceral illness also produces a conditioned avoidance of novel tastes that are paired with its presence (15).

Thus, we compared the effects of both peripheral and
TABLE 1

Macronutrient composition of low-fat, high-fat, and regular diets by kilocalories per gram and percentage of total kilocalories

<table>
<thead>
<tr>
<th>Macronutrients</th>
<th>Regular diet</th>
<th>Low-fat diet</th>
<th>High-fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (kcal/g)</td>
<td>0.8</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Carbohydrate (kcal/g)</td>
<td>2.1</td>
<td>2.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Fat (kcal/g)</td>
<td>0.5</td>
<td>0.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Total (kcal/g)</td>
<td>3.4</td>
<td>3.6</td>
<td>4.4</td>
</tr>
<tr>
<td>Protein (% of total kcal)</td>
<td>23</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Carbohydrate (% of total kcal)</td>
<td>62</td>
<td>75</td>
<td>45</td>
</tr>
<tr>
<td>Fat (% of total kcal)</td>
<td>15</td>
<td>11</td>
<td>41</td>
</tr>
</tbody>
</table>

central C75 administration to those of LiCl on their ability to reduce NaCl intake and produce a conditioned taste aversion (CTA), and we assessed the metabolic consequences of peripheral C75 to alter energy expenditure.

RESEARCH DESIGN AND METHODS

Animals. Male Long-Evans rats that weighed 310–330 g at the onset of the experiments were individually housed in Flexiglas tubs and maintained on a 12:12-h light-dark cycle in a temperature-controlled room. Animal procedures were approved by the Internal Animal Care and Use Committee at the University of Cincinnati. Rats were maintained on ad libitum food and tap water unless otherwise noted. One cohort of rats was given intraperitoneal IP injections, and in another cohort, cannulas were implanted and aimed at the i3vt. Coordinates were on the midline, 2.2 mm posterior to bregma, and 7.5 mm ventral to dura, with bregma and lambda at the same vertical coordinate (16). After 10 days of recovery, accuracy of i3vt placement was verified by i3vt infusion of 10 ng of angiotensin II in saline. Only animals that drank at least 5 ml in 1 h were used in the experiments.

Drugs. C75 was a gift from Procter & Gamble Pharmaceuticals (Cincinnati, OH). All substances were dissolved in RPMI (Gibco, Carlsbad, CA), which served as the control solution. Solutions were administered through i3vt in a 1-μl volume; solutions were injected intraperitoneally in a volume of 1 ml/kg.

Diets. Animals were maintained on pelleted rat diet (Harlan Teklad, Indianapolis, IN) in experiments 1 and 3. High-fat pelleted diets prepared by Dyets (Bethlehem, PA) were used in experiments 2 and 4. The macronutrient composition of these diets is given in Table 1.

Experiment 1: effect of IP C75 on food intake. Animals were assigned to one of six treatment groups (n = 5/group) matched by baseline body weight and food intake. On the test day, food was removed 4 h before lights off. One hour before lights off, each rat received an IP injection of 3, 10, 15, or 30 mg/kg C75 or an equal volume of saline or RPMI (the vehicle for C75). Five minutes before lights off, food was returned to the cages and intake was measured after 2, 4, and 24 h (data were similar across all time points; therefore, only the 24-h data are shown). Water was available at all times. In addition, a separate cohort of rats was maintained with ad libitum access to a high-fat diet for 3 weeks. They were then matched by average daily food intake and body weight and assigned to one of four groups (n = 5/group) to receive IP saline, RPMI, or 3 or 10 mg/kg C75. The procedure on the test day was identical to that in experiment 1.

Experiment 2: effect of IP C75 on food intake. Animals were assigned to one of six treatment groups (n = 8 or 9/group) matched by baseline body weight and food intake. On the test day, food was removed 4 h before lights off. One hour before lights off, each rat received an IP injection of 3, 10, 15, or 30 mg/kg C75 or an equal volume of saline or RPMI. Five minutes before lights off, food was returned and intake was measured after 2, 4, and 24 h. Water was available at all times. An additional cohort of animals was maintained on the high-fat diet for 10 weeks, at which time they had a significantly higher body weight than rats fed the regular diet; therefore, all animals were matched for baseline food intake and body weight. The two groups received either IP saline or IP C75 (3 mg/kg). The two groups were then assigned to one of two treatment groups (n = 6/group) matched by daily food intake and body weight. The two groups received either IP 8 mg/kg C75 or 3 mg/kg C75. On the test day, an identical procedure as described above was followed.

Experiment 3: sodium intake after IP C75. Rats avidly ingest NaCl solutions after they have been made sodium-deicient (14). However, this consumption is reduced after the administration of emetic or toxic agents such as LiCl (15). For assessing whether C75 is aversive and therefore would reduce sodium intake after sodium depletion, rats (n = 24) were given access to a 0.5-mol/l NaCl solution in a water bottle for 7 days. Water was available ad libitum in a second bottle. On the next day, the bottle with the NaCl solution and the hopper with regular diet were removed and clean food hoppers containing sodium-free rat diet (ICN Biochemicals, Cleveland, OH) were available instead (along with one water bottle). The rats were then given two subcutaneous injections of sodium chloride (2 ml/kg) 2 h apart (17). Diuresis (and presumed sodium depletion) was confirmed by observing at least 18 g of weight loss in the 3 h after injection. Twenty-four hours after the first furosemide injection, rats were given an IP injection of isonicotic LiCl (a volume equivalent to 2% of the rat’s body weight), an equal volume of isotonic saline, or 15 mg/kg C75. Fifteen minutes later, two bottles were made available in the home cages, one containing 0.5 mol/l NaCl and the other containing distilled water. Intake was measured every 30 min for 2 h.

Experiment 4: sodium intake after IP 3% and C75. Rats (n = 8/group) were treated comparably as in experiment 3, except that they were given LiCl (IP), i3vt RPMI, or i3vt C75 (15 μg).

Experiment 5: IP C75 and CTA. If rats are made ill after consumption of a novel flavor, then they avoid that flavor when encountered again, a phenomenon called CTA (15). LiCl is commonly used as a toxic agent to produce a CTA (18). The degree to which rats avoid a treatment-flavored protein can be used as an index of the aversive consequences produced by the treatment (e.g., LiCl) (e.g., [12]). Rats (n = 8/group) were assigned to one of three groups, matched for previous food intake and body weight. During phase 1, water was replaced with flavor 1 (artificially sweetened grape or cherry Kool-Aid, counterbalanced across rats and groups). Flavor stimuli were nonsweetened Kool-Aid brand grape and cherry mixes prepared according to packet instructions except that twice the amount of water was added in addition to an artificial, noncaloric sweetener (sodium saccharin 0.15%). Flavor 1 was available ad libitum for 5 days, during which time body weight and food and fluid intake were recorded. At the end of phase 1, flavor 1 was replaced by tap water for 48 h until phase 2 began. In phase 2, the rats received an IP injection of LiCl, RPMI, or 15 mg/kg C75. The rats were given ad libitum access to the alternate flavor of Kool Aid (flavor 2) instead of water immediately before the injection for 24 h. Body weight and food and fluid intake were recorded daily throughout. In phase 3 (the test phase), all rats were given 24-h access to both flavor 1 and flavor 2 without access to water. Intake of both flavors was recorded after 24 h.

Experiment 6: i3vt C75 and CTA. Rats that were matched for intake and weight before the first day of the experiment (n = 6/group) were assigned to one of two groups receiving IP 15 mg/kg C75 or an equal volume injection of the vehicle RPMI. Animals were habituated to indirect calorimeter chambers for 7 days before the test day. On the test day, animals received an injection of C75 or vehicle 1 h before lights off and food was withheld. Immediately before lights off, animals were placed in indirect calorimeter chambers with access to water only. Heat expenditure was measured during the next 24 h.

Data analysis. For multiple group designs, the data were analyzed by one-way ANOVA with post hoc tests. For designs with only two groups (e.g., experiment 5), statistical validation was assessed with unpaired t-tests. Experiment-wise significance was set at P < 0.05, two-tailed.

RESULTS

Experiment 1: effect of IP C75 on food intake. IP administration of C75 dose-dependently reduced 24-h food intake and body weight relative to both saline and RPMI (Fig. 1). Similar trends were observed after 2 and 4 h (data not presented). ANOVA revealed a reliable main effect of drug for both 24-h food intake (F(5,24) = 34.93, P < 0.01; Fig. 1A) and body weight change (F(5,24) = 8.25, P < 0.01; Fig. 1B). Tukey’s post hoc tests revealed that 10 mg/kg C75 reduced food intake relative to saline, RPMI, and 3 mg/kg C75 (all P < 0.05). Doses of 15 and 30 mg/kg C75 reduced food intake to a greater extent than 10 mg/kg (both P < 0.05). Post hoc tests also revealed that 10, 15, and 30 mg/kg C75 reduced body weight relative to the change caused by saline, RPMI, and 3 mg/kg C75 (P < 0.05) and that 15 and 30 mg/kg resulted in a significant reduction in food intake relative to 10 mg/kg C75 (P < 0.05). No other significant effects were found. In addition, relative to...
saline, IP C75 dose-dependently reduced food intake and body weight in rats maintained on a high-fat diet. ANOVA revealed a main effect of drug for kilocalories consumed during 24 h ($F_{(3,16)} = 7.14, P < 0.01$) as well as for body weight change ($F_{(3,16)} = 4.81, P < 0.05$). Subsequent Tukey’s post hoc tests revealed that 10 mg/kg C75 decreased food intake ($P < 0.05$) and body weight ($P < 0.05$) relative to all other treatments. No other significant differences were found.

**Experiment 2: effect of i3vt C75 on food intake.** i3vt C75 dose-dependently reduced 24-h food intake and body weight relative to both saline and RPMI (Fig. 2). ANOVA revealed a reliable main effect of drug for food intake ($F_{(5,36)} = 6.07, P < 0.01$; Fig. 2A) and body weight change ($F_{(5,36)} = 37.91, P < 0.01$; Fig. 2B). Tukey’s post hoc tests revealed that all doses of C75 (3, 10, 15, and 30 μg) reduced food intake and increased weight loss relative to the effects of saline and RPMI ($P < 0.05$). For weight loss, 10 and 15 μg caused a significantly greater reduction than 3 and 30 μg ($P < 0.05$). In addition, relative to i3vt administration of RPMI, 10 μg of C75 reduced 24-h food intake and body weight of rats maintained on a high-fat diet (data not shown). Similar trends in food intake were observed after 2 and 4 h (data not shown). Tukey’s post hoc tests revealed that the 10-μg dose of C75 reliably reduced 24-h food intake ($t_{(12)} = 4.76, P < 0.01$) and body weight ($t_{(12)} = 5.10, P < 0.01$).

**Experiment 3: sodium intake after IP C75.** LiCl suppressed intake of a 0.5-mol/l NaCl solution in sodium-deficient rats (Fig. 3A). IP C75 reduced 0.5 mol/l NaCl intake by 56.4% relative to RPMI. For assessing the statistical validity of these results, $t$ tests compared NaCl consumption after LiCl or C75 with intake after saline or RPMI, respectively. Both LiCl and C75 reliably reduced NaCl consumption relative to their respective vehicle (both $P < 0.05$).

**Experiment 4: sodium intake after i3vt C75.** i3vt C75 did not reliably change intake of a 0.5 mol/l NaCl solution relative to intake after i3vt RPMI. Whereas the ANOVA revealed only a near-significant main effect of treatment ($F_{(2,18)} = 3.38, P = 0.057$; Fig. 3B), subsequent Tukey’s post hoc tests revealed that LiCl reliably reduced NaCl consumption, relative to both RPMI and C75, whereas C75 did not differ from RPMI.

**Experiment 5: IP C75 and CTA.** Preference ratios were first calculated for each rat (i.e., flavor 1 intake/([flavor 1 + flavor 2 intake])). As depicted in Fig. 4A, IP C75 (15 mg/kg) reduced the preference ratios relative to what occurred after IP RPMI after both 4 and 24 h. $T$ tests revealed that
both reductions were statistically significant ($F_{(1,14)} = 3.41, P < 0.01$).

**Experiment 6: i$\text{3vt}$ C75 and CTA.** I$\text{3vt}$ C75 did not reduce 4- or 24-h preference ratios relative to i$\text{3vt}$ RPMI (Fig. 4B). That is, rats did not avoid the Kool-Aid flavor associated with either the i$\text{3vt}$ C75 or the RPMI injection. No statistically reliable differences were found between groups at either time point (both $P > 0.5$).

**Experiment 7: IP C75 and heat expenditure.** IP C75 (15 mg/kg) significantly reduced heat expenditure compared with vehicle-treated controls (Fig. 5) during the first 12 h ($F_{(1,14)} = 11.72, P < 0.05$). During the second 12 h, there were no significant differences in heat expenditure between the two groups ($P > 0.05$). These data are consistent with previous reports for LiCl (10), a known aversive agent. However, our data are not consistent with previous reports for FAS inhibitors. Makimura et al. (10) and Thupari et al. (19) reported that IP cerulenin and C75 significantly increased heat expenditure when compared with vehicle in the mouse.

**DISCUSSION**

There are several important findings from this series of experiments. First, both central (i$\text{3vt}$) and peripheral (IP) administration of C75 reduced food intake in rats, with larger doses suppressing intake to a greater extent. Consistent with this effect on food intake, body weight was also reduced by C75. Moreover, the dose of C75 required to reduce food intake when delivered through i$\text{3vt}$ was 10 $\mu$g, whereas the dose required when delivered intraperitoneally was 10 mg/kg. I$\text{3vt}$ injection resulted in greater reductions in body weight than IP doses that resulted in similar food intake reductions.

Adult mammals do a masterful job of matching caloric intake to caloric expenditure to maintain constant levels of stored fuels in the form of triglycerides in adipocytes (20–23). Although the role of FAS has been studied extensively in liver, heart, adipose tissue, and muscle (24), its role in the adult brain is essentially unknown. Whereas brain circuits that control food intake are known to be sensitive to manipulations of glucose utilization (25,26), the possibility that the fatty acid synthase pathway is also involved is a novel concept. The observation that inhibition of FAS elicits such potent changes in ingestive behavior has opened the possibility that other metabolic pathways and sensing mechanisms might be important within the brain. Historically, i$\text{3vt}$ administrations of pharmacological agents have been used to compare peripheral and CNS action of a given compound. However, they are a poor way to determine the critical site of action within the CNS because injections of compounds into cerebral ventricles will distribute compound to areas well beyond the hypothalamus (as reviewed by Grill and Kaplan [27]). Thus, the present data are consistent with the hypothesis that the hypothalamus represents a critical site of action.
for C75, but it cannot be ruled out that other areas of the CNS contribute importantly to its anorexic effect.

A reduction of food intake after the administration of a drug provides a positive indication for a potential treatment strategy for obesity. However, caution is warranted in the interpretation of any such finding because reduced food intake is also a symptom of motor impairment and/or visceral illness. Neither we nor other investigators (9,10,28) have observed obvious motoric effects of C75. However, visceral illness is more difficult to assess in rats, which are not capable of emesis. We therefore assessed the possibility that the anorexia elicited by C75 is accompanied by visceral illness by comparing the actions of C75 with a sublethal dose of the toxin LiCl. Importantly, LiCl reduces acute food intake (29) and in humans results in reports of nausea (30). In rats, LiCl reduces need-induced NaCl intake and produces CTAs, and both measurements have been commonly used as an index of visceral illness (14,15).

Both assessments of visceral illness revealed a consistent and important dissociation between IP and i3vt C75. Whereas both routes of administration reduced food intake, IP C75 elicited apparent illness and an equi-anorexic dose given through the i3vt did not. That is, an IP dose of C75 that reduced food intake by 33% reduced NaCl intake and induced a CTA. Contrary to this, i3vt C75 at a dose that reduced food intake by 33% neither reduced NaCl intake nor supported a CTA. The obvious conclusion is that the reduction of food intake elicited by i3vt C75 is not secondary to visceral illness. However, the anorexia observed in these experiments after IP C75 could be secondary to the presence of illness. Consistent with the hypothesis that IP C75 produces visceral illness, IP injection of C75 also reduced total energy expenditure like LiCl. These effects may limit the utility of peripheral FAS inhibition in the treatment of obesity.

The reduction in energy expenditure observed after peripheral C75 is particularly surprising given the reported results from Makimura et al. (10) and Thupari et al. (19), where IP cerulenin or C75 increased energy expenditure in mice. Several differences in methodology could account for the discrepant results. First, our data were collected in rats, whereas both of the other reports are from mice. Mice have higher basal metabolic rates and have different thermoneutrality temperatures than do rats. The possibility that the effects of FAS inhibition on energy expenditure interact with these species differences needs further experimental attention. Second, both of the mouse studies used repeated peripheral dosing and food was available in the indirect calorimeter during the assessment of energy expenditure. In our study, we used a single dose of C75 that we had already determined would potently suppress food intake for 24 h under the same metabolic conditions. Moreover, we withheld food during this period and it is feasible that the metabolic effects of FAS inhibition depend on ongoing nutrient availability from the gastrointestinal tract. The possibility that basal metabolic rate and/or nutrient flux contribute to the actions of C75 to alter energy expenditure will also need to be addressed in future experiments.

The present data indicate that there is a potent and specific mechanism whereby C75 reduces food intake via actions in the CNS. Such data are consistent with the hypothesis that metabolic pathways in the CNS, in neurons and/or glia, are sensitive to activity in FAS-related metabolic pathways. Several reports have suggested that increased malonyl-CoA present when FAS is inhibited is critical to its anorexic effects (9). Alternatively, C75 could produce an increase in long-chain fatty acids that have important signaling properties. For example, recent data from Rossetti and colleagues (31) demonstrated that oleic acid injection directly into the CNS can reduce food intake and hepatic glucose output. These findings raise the possibility that there are mechanisms by which long-chain fatty acids can provide a signal of “nutrient abundance” to specific areas of the brain and that the brain then directs changes in fuel utilization from carbohydrates to lipids by limiting further entry of exogenous (food intake) and endogenous (hepatic glucose output) nutrients into the circulation (31). Although the current data do not directly address these two possible mechanisms, the picture that emerges from all of these reports is a critical role for fatty-acid metabolism in the CNS to influence both food intake and peripheral energy utilization (32). This is counterintuitive because the CNS has long been regarded as an organ that typically utilizes glucose and not fatty acids as its primary fuel. Nevertheless, these data open up the possibility that selective FAS inhibition in the CNS may be a viable strategy to treat obesity.

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REFERENCES

18. Nachman M, Ashie JH: Learned taste aversions in rats as a function of dosage, concentration, and route of administration of LiCl. *Physiol Behav* 10:73–78, 1973