Cholecystokinin and serotonin receptors in the regulation of fat-induced satiety in rats

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The present study investigated the relationship between endogenous CCK and serotonin (5-HT) in fat-induced satiety. Male Wistar rats with duodenal cannulas were adapted to eating 6 h/day along with receiving an infusion of saline or one of two isocaloric solutions (10 ml, 0.45 ml/min) varying in fat and carbohydrate content (20 or 80% energy from fat). Rats were infused 10 min after food presentation. The satiation/satiety response was determined from measures of meal size (MS), intermeal interval (IMI), and total food intake (TFI). Infusion with either fat solution reduced MS compared with saline; however, the 80% fat infused reduced TFI and lengthened the IMI compared with saline and the 20% fat infused. CCK and 5-HT involvement in fat-induced satiety was investigated by preceding the 80% fat infusion with CCK and/or 5-HT; receptor antagonists Devazepide (Dev) and Tropisetron (Trop). A CCK releaser, trypsin inhibitor (TI), was added to the 20% fat infusion to enhance satiety. Pretreatment with Dev or Trop alone attenuated the inhibitory effects of the 80% solution on IMI, whereas reversal of the inhibitory effects on MS and TFI were sensitive only to Dev at the doses provided. Both antagonists together completely blocked the satiating effects of the 80% fat infusion on IMI, whereas reversal of the inhibitory effects on MS and TFI were sensitive only to Dev at the doses provided. Addition of TI to the 20% fat infusion lengthened the IMI but did not affect MS or TFI. These results provide evidence for the participation of both endogenous CCK and 5-HT in the satiety response to fat in the intestine.

GASTRIC DISTENSION and the rate of gastric emptying have traditionally been regarded as among the primary regulators of short-term food intake. However, studies using the sham-feeding paradigm combined with intestinal nutrient infusion have raised questions about the relative importance of gastric mechanisms. This paradigm is unique in that influences of gastric emptying and gastric distension as controls of food intake are eliminated because orally consumed food drains directly out of the stomach via an open gastric fistula (30). A number of studies have shown that animals with gastric fistulas (sham fed) stop eating when nutrients, such as fat, are infused into the small intestine (16, 17, 30, 35, 36). Behaviors associated with satiety typically accompany intestinal fat infusions, indicating that the suppression of food intake in response to the infusion is due to a satiety effect rather than a toxic or aversive effect (1, 19).

In addition to cessation of eating, satiety behaviors usually persist for several hours after a meal has been consumed. Gastric distension is transient in nature because relief begins as soon as food begins to empty from the stomach into the small intestine (24). Thus alternative, possibly intestinal or postabsorptive mechanisms are critical in sustaining satiety. Whereas the satiating effect of some nutrients may depend on postabsorptive mechanisms, the satiating effect of fat appears to depend on preabsorptive (intestinal) satiety-related mechanisms. Studies in humans and animals have shown that intravenous infusions of emulsified lipids are less satiating than intraintestinal infusions of lipid (18, 20, 32, 36). Additionally, work in our laboratory has demonstrated that fat, present in the upper small intestine, reduces food intake and prolongs postprandial satiety in normal (nonsham) feeding rats (6, 7). Collectively, these studies suggest that fat interacts at preabsorptive intestinal receptor sites to induce satiety. This response to fat may be mediated by the release of chemical modulators that signal satiety. Two candidates for this role include the peptide CCK and the indolamine serotonin (5-HT).

The gastrointestinal tract is rich in endocrine and neuronal cells that synthesize and secrete CCK and 5-HT (10, 14). Both CCK and 5-HT are secreted in response to intraluminal stimuli associated with ingestion of a meal, and both have been shown to reduce food intake and elicit behaviors associated with satiety when exogenous sources are administered to rats. Accordingly, attention has been focused on these two substances as potential signals from the gut to the brain in the control of short-term food intake. Numerous studies have taken advantage of the various advances in the pharmacology of CCK and 5-HT to investigate the physiological significance of CCK and 5-HT in feeding. Peripheral and central administration of exogenous sources of CCK and 5-HT along with the use of specific receptor antagonists, agonists, and reuptake inhibitors have provided valuable evidence for the involvement of both CCK and 5-HT in short-term food intake. However, little is known about the interconnection between endogenously released CCK and 5-HT in mediating the satiation/satiety response to dietary nutrients such as fat. The purpose of the present study was to use selective antagonists to investigate the roles of endogenous CCK and 5-HT acting at CCK type A and...
5-HT type 3 receptors in fat-induced satiation and satiety.

**METHODS**

Animals and surgical preparation. The study was approved by the Animal Use and Welfare Committee at the University of California, Davis. Six male Wistar rats (Simonsen, Gilroy, CA) weighing 275–300 g each were surgically equipped with chronic duodenal cannulas as described previously (7). Briefly, rats were anesthetized (ketamine, Rompun, acepromazine, 50:5:0.75 mg/kg body wt) and the abdomen was opened via a midline incision. Silastic cannula tubing (inner diameter 0.025 in.; Silastic biomedical grade tubing, Dow Corning, Midland, MI) was inserted and secured in the duodenum distal to the pancreatic duct and proximal to the ligament of Treitz. The tubing was exteriorized mesocapsular-ly and threaded through a coil spring that was attached to a swivel outside of the cage. This arrangement allows rats relatively free movement in their cages and permits daily infusion of treatments without handling or disturbing the animals. Rats were allowed at least 1 wk for postoperative recovery. During the recovery period, rats were infused daily with 3–5 ml physiological saline to ensure that the cannulas remained patent. Rats were housed in hanging wire bottom cages modified to allow computer analysis of food intake and feeding patterns.

Diet. Rats were fed an elemental diet containing (in g/kg) 320 sucrose, 320 cornstarch, 180 l-amino acid mix (Dyets no. 510016), 100 α-cellulose, 60 mineral mix (29), and 20 vitamin mix (6, 29). This type of diet minimizes CCK release because intact protein and fat, two stimulators of CCK release (15), were not included in the diet. Therefore, both dietary fat and the potential stimulation of CCK release by fat were controlled via the infusate. Serotonin release from intestinal enterochromaffin cells is sensitive to a number of different stimuli related to feeding (33), making it difficult to minimize 5-HT release. However, the ingested diet in this study was the same across all treatments.

Infusion treatments. Two isocaloric intestinal infusates were prepared in physiological saline varying in the percent energy from fat and carbohydrate (CHO). Ten percent Intralipid (Kabi Pharmacia, Clayton, NC; gift from Clintec Nutrition, Deerfield, IL) served as the fat source, and food-grade dextrose (Dyets, Somerville, NJ) served as the carbohydrate source. The experimental infusates were prepared in 10-ml aliquots containing 20 or 80% energy from fat and energy balanced with energy from CHO (80 or 20%) to provide 1 kcal/ml. Physiological saline was chosen as the control infusate. Other control infusates such as an energy control (see protocol below). The role of endogenous 5-HT in fat-induced satiety was investigated by testing the effectiveness of the 5-HT3 receptor antagonist, Tropisertan (Trop; ICS-205–930; endo-1H-indole-3-carboxylic acid 8-methyl-8-azacyclo[3.2.1]oct-3-yl ester; Research Biochemicals International, Natick, MA) to prevent or reverse the satiating effects of the 80% fat infusion. A 5-HT3 receptor antagonist was chosen because of the widespread distribution of 5-HT3 receptors located onafferent and efferent visceral neurons, particularly associated with gastrointestinal viscera but also within the enteric and central nervous system (33). Trop (1 mg/kg) was dissolved in saline (2 ml) and administered following the same protocol as that used for Dev.

CCK-5-HT. To determine if a relationship exists between endogenous CCK and 5-HT in the regulation of fat-induced satiety, both antagonists, Dev and Trop, were administered together and tested for their effectiveness to reverse the satiating effects produced by the 80% fat infusion.

Dev (1 mg/kg) and Trop (1 mg/kg) were delivered together in the 0.5% methylcellulose vehicle (2 ml) via the duodenal cannula 30 min prior to infusion of the 80% fat solution. Experimental protocol. After recovery from surgery, rats were adapted to an eating regimen that allowed unrestricted access to the elemental diet for 6 h/day after an 18-h fast. In addition, rats were acclimated to the food intake-monitoring cages, a reverse 12:12-h light-dark cycle, and the infusion of 10 ml of a test solution. When total food intake (TFI) after saline infusion was similar to their presurgical (intact) intakes (7–10 days postoperative), the study was initiated.

Once the experimental period began, rats were given their food cups at 1000, which was the beginning of the dark phase of the cycle. Ten minutes later, at 1010, each rat received one of four experimental infusates [i.e., 80% fat (20% CHO), 20% fat (80% CHO), saline, or TI + 20% fat solution] through the duodenal cannula for 23 min. The 10-min preinfusion period was adequate for rats to begin eating (6). Once the infusion was finished, rats were left undisturbed until the food cups were removed at 1600. On days that rats were pretreated with the CCK antagonist, the 5-HT3 antagonist, or both antagonists together, the experimental protocol was the same as described above except antagonist(s) were administered 20 min before diet presentation or 30 min before infusion of the 80% fat solution (at 0940).

Food intake and feeding patterns were monitored throughout the 6-h experimental feeding period with a computerized food intake-analyzing system. The experiment was designed such that each animal received all of the different treatments in random order. Rats received the test solutions on more than one occasion (typically 3–5 times) to obtain a mean feeding response to each treatment. The number of days between infusions of the same test solution varied between 4 and 7 days. Rats were weighed periodically throughout the experiment to ensure appropriate growth.

Analysis. Food intake and feeding patterns were monitored and analyzed every day for 6 h. In addition, food cups were weighed manually before and after the feeding period. Vari-
variables measured included the size of the first and second meals of the feeding period, the intermeal interval (IMI) period separating the first two meals consumed, and total voluntary energy intake for the 6-h feeding period. A meal was defined as consumption of 0.5 g or more of diet with a 10-min minimum IMI between two meals. Definitions for these characteristics of the meal pattern analysis are consistent with other reports in the literature (8, 23).

Statistical analysis. The effect of saline or fat (20 or 80%) on food intake and feeding patterns and the effectiveness of Dev, Trop, Dev + Trop, and TI to reverse or enhance satiety produced by the 80 or 20% fat infusate was determined by analyzing the feeding response to each treatment per rat with repeated-measures ANOVA (26). With the use of treatment as the main effect and rat as the blocking variable, significant differences among treatment means (adjusted) were analyzed by pairwise t-test for appropriate comparisons. Statistical significance was assumed for the t-tests and the ANOVA when computed P values were <0.05. Statistical calculations were computed using PC-SAS general linear model procedure.

RESULTS
Sizes of meal 1 and meal 2. Table 1 illustrates the effect of the various treatments on the first two meals consumed by the rats in the 6-h feeding period. The size of meal 1 differed among treatments [F (6,169) = 13.24, P < 0.0001], whereas the size of meal 2 did not. Infusion with either nutrient-containing solution alone resulted in significantly lower first-meal intakes compared with saline infusion (P < 0.001). Dev (1 mg/kg) treatment alone 30 min before infusion of the 80% fat solution increased the size of the first meal (62%) compared with the 80% fat solution alone, resulting in a first meal size (MS) that was similar to that resulting from saline. Although Trop did not significantly increase the size of meal 1, Trop enhanced the effect of Dev on first-meal intakes by 34% compared with Dev alone (P < 0.005). Addition of TI, a potent CCK releaser, to the 20% fat solution did not reduce first MS from saline. Although Trop did not significantly alter the IMI (data not shown).

Table 1. MS, IMI, TFI, and total energy intake in infused rats

<table>
<thead>
<tr>
<th>Infusate Treatment</th>
<th>Meal 1</th>
<th>Meal 2</th>
<th>IMI, min</th>
<th>TFI, g</th>
<th>Total Energy Intake, kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>80% Fat</td>
<td>2.26 ± 0.21*</td>
<td>3.26 ± 0.36</td>
<td>173 ± 8.8§</td>
<td>8.54 ± 0.50*</td>
<td>38.1 ± 1.82*</td>
</tr>
<tr>
<td>80% Fat + Dev</td>
<td>3.67 ± 0.32‡</td>
<td>3.60 ± 0.39</td>
<td>83.3 ± 9.8‡</td>
<td>11.9 ± 0.52‡</td>
<td>49.3 ± 2.97‡</td>
</tr>
<tr>
<td>80% Fat + Trop</td>
<td>1.99 ± 0.26*</td>
<td>3.00 ± 0.33</td>
<td>100.6 ± 9.6†</td>
<td>9.50 ± 0.60‡</td>
<td>41.4 ± 1.36*</td>
</tr>
<tr>
<td>80% Fat + Dev + Trop</td>
<td>4.92 ± 0.37†</td>
<td>2.84 ± 0.36</td>
<td>66.8 ± 8.5‡</td>
<td>15.6 ± 0.41†</td>
<td>61.5 ± 2.54†</td>
</tr>
<tr>
<td>Saline</td>
<td>4.31 ± 0.79‡‡</td>
<td>3.32 ± 0.40</td>
<td>40.6 ± 5.3*</td>
<td>15.1 ± 0.46†</td>
<td>49.9 ± 1.14‡</td>
</tr>
<tr>
<td>20% Fat</td>
<td>1.65 ± 0.37*</td>
<td>3.67 ± 0.38</td>
<td>61.6 ± 7.0‡</td>
<td>13.1 ± 0.53‡</td>
<td>53.3 ± 2.20‡</td>
</tr>
<tr>
<td>20% Fat + TI</td>
<td>2.35 ± 0.27*‡</td>
<td>3.47 ± 0.30</td>
<td>100.8 ± 6.8†</td>
<td>12.9 ± 0.56‡</td>
<td>52.5 ± 1.76†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 rats. Fat infuses (10 kcal each) contained either 80 or 20% energy from fat and were balanced for energy with carbohydrate (CHO) (20 or 80% energy from CHO, respectively). Pretreatment with Devazipide (Dev) and Tropisetron (Trop), alone or together (1 mg/kg each), was 30 min before infusion of 80% fat solution. Trypsin inhibitor (TI; 200 mg) was added directly to 20% fat solution. MS, meal size; IMI, intermeal interval; TFI, total food intake. Values with different symbols indicate significant differences (P < 0.05) between treatments for respective columns.
observed when both antagonists were administered together before saline infusion (not significant, data not shown).

**DISCUSSION**

Both CCK and 5-HT have been implicated independently as factors affecting food intake. Peripheral administration of CCK or 5-HT suppresses food intake and elicits the behavioral sequence of satiety (3, 36). The presence of lipids in the small intestine results in similar inhibitory effects on food intake and behavior associated with satiety (6, 7, 19). Studies utilizing CCK type A receptor antagonists suggest that CCK is involved in MS reductions induced by fat (21, 22, 37). The involvement of endogenous CCK in fat-induced postprandial satiety has not been demonstrated. Additionally, the role of fat-stimulated release of endogenous 5-HT in satiation and satiety is not known. The aim of the present study was to address these questions.

Three variables of feeding were used to define satiation and satiety: MS, IMI, and TFI. Measurements of MS provide information about within-meal satiety (i.e., satiation), which occurs as a consequence of feeding, while the IMI reveals information about the postprandial effect of the meal. TFI for the 6-h period provides information about compensatory alterations in food intake due to treatment. Analysis of these variables in combination reveals information about the sequence or pattern of feeding activity (4). Furthermore, transitions in feeding patterns after specific experimental manipulation can provide evidence for the mechanism(s) underlying the processes of satiation and satiety. Fat has a significant role in satiation and satiety, and results from the present study indicate that both CCK and 5-HT are involved in mediating the inhibitory effects of intestinal fat on feeding behavior.

Two approaches were used to investigate the involvement of CCK in fat-induced satiety. One approach consisted of testing the effectiveness of a potent CCK antagonist to block the effects of a fat solution (80% fat) known to have potent satiating effects. The second approach reversed the paradigm and tested the effectiveness of a CCK releaser, TI, to enhance the satiating capacity of a low-fat solution (20% fat) known to have a lower satiety/satiating potency than its opposing higher fat treatment. The results obtained from the two approaches support a role for endogenous CCK in fat-induced satiety. However, Dev blocked the suppressive effect of the 80% fat solution on meal 1, whereas TI did not significantly affect the size of the first meal. The lack of an effect on meal 1 by TI was anticipated, because infusion with the 20% fat solution without TI reduced the size of the first meal to 62% of the saline treatment, which did not differ from the suppressive effect of the 80% fat solution on meal 1. Therefore, it is likely that the initial release of CCK by the 20% fat solution was sufficient to terminate feeding and reduce MS, and any additional release did not enhance the effect. The effect of TI on satiety was demonstrated by prolonging the period of postprandial satiety, which was 40% longer than after treatment with 20% fat alone. A previous study suggested that treatments that are likely to prolong the release of CCK will have a greater satiety-inducing effect (7). Augmentation of CCK release by TI may lengthen the interval before its clearance from plasma and thus lengthen IMI. Although postprandial satiety in response to the 20% fat infusate was enhanced by the addition of TI to the infusate, TFI was not affected. In contrast, the treatment with Dev partially reversed the suppressive effect of the 80% fat infusate on total 6-h food intake. The explanation for this difference is not clear but may reflect mechanistic differences of fat and TI stimulation of CCK release; the operation of other mechanisms involved in regulating energy intake that may be sensitive to intraluminal fat but not TI; and/or variability in activity of CCK after fat, TI, and Dev treatment.

The control of short-term food intake is comprised of a network of interactions that produce feeding behaviors described as satiation and satiety. A specific interaction between CCK and 5-HT in the control of food intake has been suggested by experiments utilizing exogenous CCK, 5-HT, and D-fenfluramine, as well as CCK and various 5-HT receptor antagonists (3). Simultaneous blockade of CCK-A and 5-HT3 receptors in our study supports a physiological interaction between CCK and 5-HT systems in the satiety-inducing effects of intestinal fat. Co-administration of Dev and Trop, 30 min before infusion of the 80% fat solution, abolished the effects of the high fat infusate on all feeding variables measured. These results, taken together with feeding data after administration of either CCK or 5-HT antagonist alone, indicate that MS, IMI, and TFI are influenced by both physiological systems.

In 1990, Cooper and Dourish (9) proposed a model based on the interactions of exogenous CCK and 5-HT for determining within-meal satiation, as measured by MS. The model predicts that elevation of CCK and 5-HT will trigger mechanisms that result in meal termination, and blocking either component will consequently affect the capacity of the other component to induce satiety. Data from the present study provide evidence for participation of endogenous CCK and 5-HT in nutrient-stimulated, within-meal satiety. Dev pretreatment increased the size of meal 1 to an intake that was 85% of meal 1 intake in saline-treated rats, which was near complete reversal of the inhibitory effects of the 80% fat solution on MS. Trop pretreatment increased the size of meal 1 less effectively than Dev, resulting in first meal intakes that were 47% of the saline control. The observation that Trop, a selective 5-HT3 receptor antagonist, was less effective than Dev in increasing meal 1 is consistent with reports that systemically administered 5-HT mediates its effect on MS primarily through 5-HT3 and/or 5-HT2 receptors rather than 5-HT3 Receptor activation (27, 31). While this explanation is acceptable for interpreting the present results, the participation of endogenous 5-HT at 5-HT3 receptors in mediating feeding behavior cannot be ignored. Administration of both 5-HT and CCK receptor antagonists together increased the size of the first meal to 114% of saline intakes. This enhanced
The action of endogenous 5-HT at 5-HT_3 receptors in mediating within-meal satiation appears relatively weak compared with CCK-A receptor activation by CCK. In contrast, 5-HT_3 receptor activation by 5-HT seems equally important as CCK activity in mediating the postmeal nonfeeding period of satiety (postprandial satiety measured by IMI). Separately, Dev and Trop shortened the IMI by ~50%, which was significantly shorter than the IMI after infusion with the 80% fat solution alone but still significantly longer than IMI after saline infusion. Together, however, Dev and Trop treatment shortened the length of the IMI to one that was similar to saline. The additive effects of Dev and Trop on IMI suggest that both CCK and 5-HT are essential in maintaining postprandial satiety. The additive effects under the experimental conditions of this study argue that CCK and 5-HT act via separate mechanisms to influence postprandial satiety. The interpretation of these results agrees with other studies suggesting that satiety-related CCK and 5-HT mechanisms are dissociable (11–13). To the best of our knowledge, this is the first demonstration of a potential physiological interaction between CCK and 5-HT acting on 5-HT_3 receptors in postprandial satiety. Thus the sequence of satiety (within-meal and postprandial effects) does not appear to be fully expressed unless both cholecystokininergic and serotonergic systems are active.

In addition to within-meal and postprandial effects, subsequent food intake and total energy intake are important components of the satiety response. TFI is comprised of all the meals taken in a feeding period and reveals information about compensatory feeding behavior that may be related to changes in feeding patterns experienced earlier in the feeding cycle. TFI measurements after fat treatment alone or in combination with antagonist pretreatment followed similar response patterns observed with first-meal intakes. Dev was more effective than Trop in relieving the suppressive effects of the 80% fat solution, but administered together Dev and Trop resulted in a total energy intake that was 123% of intakes after saline treatment. A synergistic relationship between CCK and 5-HT mechanisms to control TFI is suggested by these results.

The results from the present study support the hypothesis that endogenous CCK and 5-HT are involved in mediating the physiological satiating effects of intestinal fat. However, the observation that the antagonists had little impact on feeding behavior when administered before saline infusion may present confusion in the interpretation of these results. Theoretically, the antagonists should stimulate feeding in saline-infused rats so that larger meals are consumed and eating occurs more frequently, because the satiating effects of endogenous CCK and 5-HT at baseline levels would be eliminated. At least one exception to this idea is when the background motivation to eat is high (e.g., extended period of food deprivation), in which case stimulation of feeding above baseline or control levels is not always apparent (reviewed in Ref. 34). In the present study, rats were deprived of food for 18 h and thus highly motivated to eat. The lack of stimulated feeding above saline control levels with antagonist pretreatment was anticipated and should not detract from the results obtained with nutrient infusions plus antagonists and/or agonists, which support CCK and 5-HT systems as fundamental in the network of interactions that control short-term food intake.

This study combined expertise and experimental techniques from a number of disciplines to investigate the roles of CCK and 5-HT in mediating the satiating/satiety effects of intestinal fat. Computer monitoring of feeding and meal pattern analysis was essential for identifying the operation of CCK and 5-HT mechanisms in response to fat on IMI (within-meal satiation), IMI (postprandial satiety), and total energy intake. Dev pretreatment reversed the inhibitory effect of the 80% fat infusate on first-meal intakes and partially reversed the effect of the 80% infusate on IMI and TFI. Addition of T1 to the 20% fat infusate enhanced its capacity to sustain satiety, as measured by a 40% IMI extension. Trop did not have a significant effect on first-meal intakes or total energy intake at the doses provided, but partially blocked the effects of the 80% fat infusate on IMI. These data provide evidence that the processes that articulate feeding behavior in response to fat are activated by cholecystokininergic and serotonergic mechanisms. Furthermore, these data emphasize that a single physiological mechanism does not account for all the alterations in feeding behavior associated with satiation and satiety, although they support an important physiological role for both CCK and 5-HT in the control of short-term food intake.

Perspectives

Dietary-induced signals of satiation and satiety have been investigated for many years; however, the role of the macronutrients, especially fat, in either satiation or satiety is still unclear. Furthermore, the physiological mechanisms underlying nutrient-induced satiation and satiety remain to be elucidated. This study has demonstrated that, in an experimental design combining nutrition, pharmacology and physiology while monitoring and analyzing food intake and feeding patterns, CCK and serotonin are key mechanisms governing satiation and satiety. In addition, the impact each system has or can have at different stages in the feeding paradigm became evident. This may be useful for developing dietary strategies around fat or may suggest pharmacological approaches to maximize the positive satisfying experience of eating while discouraging consumption of excess energy.

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