5-HT3 receptors participate in CCK-induced suppression of food intake by delaying gastric emptying

Matthew R. Hayes, Rachael L. Moore, Samit M. Shah, and Mihai Covasa

Department of Nutritional Sciences, College of Health and Human Development, The Pennsylvania State University, University Park, PA, 16802-6504

Address correspondence to: Matthew R Hayes
Department of Nutritional Sciences
College of Health and Human Development
The Pennsylvania State University
126 South Henderson
University Park, PA, 16802
Telephone: 814-863-8191
Fax: 814-863-6103
Email: mrh212@psu.edu
ABSTRACT

Serotonin type-3 (5-HT3) receptors have been shown to participate in the negative feedback control of food intake. We have previously reported that cholecystokinin (CCK)-induced suppression of food intake is partly mediated through 5-HT3 receptors when rats were tested on a preferred liquid diet, but whether such an effect occurs when tested on a solid maintenance diet is unknown. In the present study, we examined the effects of ondansetron, a selective 5-HT3 antagonist, on CCK-induced suppression of solid chow intake. Intraperitoneal administration of ondansetron significantly attenuated 30- and 60-min CCK-induced reduction of food intake, with suppression being completely reversed by 120 min. It is not known whether 5-HT3 receptors directly mediate CCK-induced satiation or whether their participation depends on CCK acting as part of a feedback cascade to inhibit ongoing intake. Since CCK-induced inhibition of sham feeding does not depend on additive gastric/post-gastric feedback signals, we examined the ability of ondansetron to reverse CCK-induced satiation in sham feeding rats. Ondansetron did not attenuate reduction of sham feeding by CCK, suggesting that ondansetron does not directly antagonize CCK-satiation signals. CCK suppresses real feeding through a delay in gastric emptying. Ondansetron could attenuate CCK-induced reduction of food intake by reversing CCK-induced inhibition of gastric emptying. We found that blockade of 5-HT3 receptors attenuates CCK-induced inhibition of gastric emptying of a solid meal, as well as saline and glucose loads. We conclude that 5-HT3 receptors mediate CCK-induced satiation through indirect mechanisms as part of a feedback cascade involving inhibition of gastric emptying.
INTRODUCTION

Gastric emptying is one mechanism involved in the regulation of food intake (33, 43). A delay in gastric emptying limits the rate of absorption by reducing the rate of nutrient delivery to the small intestine (5, 15, 58) and is associated with suppression of food intake (43). Inhibition of gastric emptying is mediated by peripheral vagal and sympathetic nerves and by the release of a variety of peptides and neurotransmitters (43, 54). Cholecystokinin (CCK) and serotonin (5-Hydroxytryptamine; 5-HT) are two humoral signals thought to exert control on gastric emptying and are released in response to nutrients entering the duodenum (6, 28, 30, 43, 49, 60). Both of these satiety signals bind to receptors on terminals of vagal afferent fibers, resulting in a delay in gastric emptying (4, 24, 49).

Considerable evidence indicates that the serotonergic system participates in the negative feedback control of food intake (see for review 25). Systemic serotonergic activity has been shown to induce an anorectic response through activation of serotonin-3 (5-HT3) receptors under a number of paradigms (1, 21). One such response is the mediation of nutrient-induced inhibition of gastric emptying (49). Like 5-HT, one mechanism by which CCK suppresses food intake is through its ability to inhibit gastric emptying (3, 12, 19, 36, 40, 43, 55).

Previous studies have shown an interaction between the serotonergic and cholecystokininergic systems in the control of food intake (7, 8, 47). While original exploration of this interaction focused on central serotonergic activity, recent reports (11), including those from our own laboratory (22), have shown that systemic administration of a selective 5-HT3 receptor antagonist attenuates CCK-induced satiation. Furthermore, peripheral 5-HT3 and CCK-A receptors cooperate interdependently in the control of CCK-induced suppression of food intake (22).

Although it is known that blockade of 5-HT3 receptors produces an attenuation of CCK-induced suppression of food intake, the underlining physiological mechanisms are still unclear. Considering that there is a gastric inhibitory contribution to the overall satiety action of CCK and that 5-HT3 receptors participate in CCK-induced suppression of food intake, it is plausible that 5-HT3 receptors participate in cholecystokininergic modulation of gastric emptying.

We (22) and others (11) have previously reported that cholecystokinin (CCK)-induced suppression of food intake is partially mediated by 5-HT3 receptors when rats were tested on a preferred sucrose solution. However, participation of 5-HT3 receptors in termination of food intake by CCK when rats consume a regular solid maintenance diet has not been investigated. Furthermore, it is not known whether 5-HT3 receptors directly mediate CCK-induced satiation or whether their participation depends on CCK acting as part of a feedback cascade to inhibit ongoing intake. Since CCK-induced inhibition of sham feeding does not depend on gastric/post-gastric feedback signals, we examined the ability of ondansetron, a selective 5-HT3 receptor antagonist, to reverse CCK-induced satiation in sham feeding rats. CCK has been shown to mediate suppression of gastric emptying by both caloric and non-caloric gastric loads. Therefore, in these studies we also examined participation of 5-HT3 receptors in CCK-induced inhibition of gastric
emptying of solid as well as nutritive and non-nutritive liquid gastric loads, a mechanism involved in the control of real feeding but not sham feeding. We report that peripheral administration of ondansetron does not alter inhibition of sham feeding by CCK, but attenuates CCK-induced inhibition of gastric emptying of solid food and liquid gastric loads.

METHODS

Subjects and Drugs:
Adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) were individually housed in hanging wire bottom cages in a temperature-controlled vivarium with ad libitum access to standard pelleted rodent chow (Purina 5001) and water except during experiments or overnight food deprivation as indicated below. The rats were maintained on a 12:12-h light-dark cycle (lights off at 1800h) and were habituated to laboratory conditions for one week before surgery or initiation of experiments. These protocols were approved by The Pennsylvania State University Institutional Animal Care and Use Committee.

The drugs used in these experiments were cholecystokinin octapeptide sulfate (CCK-8; American Peptide, Inc., Sunnyvale, CA) and ondansetron (2.0 mg/ml, Burns Veterinary Supply, Rockville, NY), a selective 5-HT3 receptor antagonist. All drugs were dissolved in sterile 0.9% saline and administered via intraperitoneal (IP) injection in a volume of 1.0 ml/kg body weight, with all rats receiving the same drug treatment on the same day. The CCK doses (1.0 and 2.0 µg/kg) were chosen based on numerous CCK-dose response studies demonstrating reliable and replicable suppression of both food intake as well as gastric emptying (9, 22). For sham feeding, CCK was administered at a dose of 2.0µg/kg to produce a percent suppression in sham feeding comparable to that seen in real feeding by CCK. The ondansetron dose of 1.0mg/kg has been used previously in experiments exploring 5-HT3 receptor mediation of CCK’s effects (11, 22).

Surgical Procedures:
Gastric cannulation.

Rats used in sham feeding studies were implanted with stainless steel gastric cannulas according to a modification of the procedure previously described by Yox and Ritter (61). Briefly, the animals were anesthetized with a Xylazine / Ketamine / AcePromazine cocktail and the flanged end of a stainless steel gastric cannula (13 mm long, 6 mm ID, 8 mm OD) was inserted through the ventral wall of the nonglandular portion of the stomach (corpus) near the greater curvature. The cannula was secured with a purse-string suture, a piece of Marlex mesh was placed around it to help prevent leaking, and the nonflanged end of the cannula was externalized through an incision in the left paramedian abdominal wall. The cannula was kept closed with a stainless steel screw, except during experiments. A minimum of 10 days was allowed for recovery from surgery.

Experiment 1: Effects of 5-HT3 receptor blockade on CCK-induced inhibition of solid food intake
Eight food deprived (17h) rats (230-260 g) were injected IP with either NaCl, ondansetron (1.0mg/kg), CCK (1µg/kg), or a combined injection of CCK (1µg/kg) and ondansetron (1.0mg/kg). Five minutes after the injection, a pre-weighed amount of standard rodent chow was presented to the rats and food intake was recorded at 0.5, 1, and 2h post injection taking into account spillage that was collected in a tray placed below the cage. Each drug treatment was separated by a minimum of 48 hours and was bracketed by a NaCl condition. Rats were tested at least twice under each condition.

Experiment 2: Effects of 5-HT3 receptor blockade on CCK-induced inhibition of sham feeding.

At the completion of Experiment 1, five rats were equipped with chronic gastric cannula and were trained to sham feed a 15% sucrose solution (w/v). Following overnight food deprivation, rats were removed from their cages, the stainless steel screw was removed from the gastric cannula, and their stomachs were gently lavaged with warm tap water (37°C). After a drainage tube was attached to the open cannula and drugs were administered, the rats were placed in Plexiglas sham-feeding boxes as previously described by Yox and Ritter (61). Intraperitoneal drug administration consisted of either NaCl, ondansetron (1.0mg/kg), CCK (2.0µg/kg), or a combined injection of CCK (2.0µg/kg) and ondansetron (1.0mg/kg). Immediately following injection, rats were presented with a calibrated glass burette filled with 15% sucrose solution (w/v) and intake was recorded every 5 min for the ensuing 60 min. Each drug treatment was separated by a minimum of 48 hours and was bracketed by a NaCl condition.

Experiment 3: Effects of 5-HT3 receptor blockade on CCK-induced suppression of solid food gastric emptying

In a separate group of 19 rats (260-450 g), we examined gastric emptying of maintenance rat chow. Following an overnight fast, rats were injected IP with either NaCl, ondansetron (1.0mg/kg), CCK (1µg/kg), or a combined injection of CCK (1µg/kg) and ondansetron (1.0mg/kg). Pre-weighed rat chow was presented 5-min later. All rats were euthanized by CO₂ asphyxiation 60-min after chow presentation. To measure the gastric contents, the stomach was exposed via a midline celiotomy, ligated at the pylorus and cardia, resected, and weighed. The resected stomach was incised and scraped of any residual test meal. The empty stomach was blotted (to remove excess liquid) and weighed. To calculate the amount of the test meal that emptied from the stomach, the remaining stomach contents were dried overnight at 100°C and were compared to the original moisture content of the chow ingested, determined by drying pre-weighed pellets overnight. Gastric emptying of ingested rat chow, expressed in grams of dry matter (DM) emptied, was determined by the following equation: Total DM emptied (g) = [(Chow Intake (g) * % DM) – (Stomach DM (g))].

Experiment 4. Effects of 5-HT3 receptor blockade on CCK-induced suppression of gastric emptying of liquid solutions.

A separate group (n = 7) of rats (300-330 g) were adapted to experimental conditions for one week prior to testing. Following an overnight fast, water was removed one hour before drug administration. At 0900 h rats received an IP injection containing either NaCl, ondansetron (1.0mg/kg), CCK (1.0µg/kg) or a single combined injection of
ondansetron (1.0mg/kg) and CCK (1.0µg/kg). Five minutes following drug administration, 5ml of 0.9% NaCl or 10% glucose solution (w/v) containing 0.006% phenol-red was instilled into the rat’s stomach via an orally inserted 8-Fr polyethylene intragastric tube. Rats were immediately returned to their homecage following gastric load. Following a five (NaCl) or ten (glucose) min emptying period, the tube was reinserted into the stomach and the remaining gastric contents were withdrawn. The stomach was rinsed repeatedly with 0.9% NaCl until withdraws were void of any visible phenol indicator. Collected volume was measured, and the gastric contents were centrifuged at 3,200 rpm for 10 min to remove any particulate matter. Gastric emptying was measured by dye-dilution spectrophotometry from absorption at 550nm. Briefly, a 1.0 ml sample from the centrifuged gastric contents was buffered with 24.5ml of 0.014 M Na₃PO₄·12H₂O. The spectrophotometric absorbance of each buffered sample was compared with that of a 1.0 ml buffered sample from the originally instilled phenol red solution to determine the volume of the original test load remaining in the stomach at the end of the emptying period. Each drug treatment was separated by a minimum of 48 h and was bracketed by a control condition. Rats were tested at least twice under each condition, with all rats receiving the same drug treatment on the same day. These experimental techniques have been detailed previously (29, 55).

Statistical Analyses

Data for each respective study were analyzed separately and expressed as mean ± SEM. Rat chow and 15% sucrose solution intakes for all time points were analyzed by two way repeated measures analysis of variance (ANOVA), with CCK and ondansetron drug treatments as the main variables. Data for liquid gastric emptying are expressed as the percentage of the liquid emptied and were analyzed by two way repeated measures ANOVA, with CCK and ondansetron drug treatments as the main variables. Comparisons between treatment means (adjusted) were analyzed by Tukey’s honestly significant difference test with P< 0.05 considered statistically significant.

Gastric emptying of rat chow was expressed in grams of dry matter emptied and was analyzed by two way ANOVA. Comparisons between the results among treatment means (adjusted) were analyzed by Student’s t-test with P< 0.05 considered statistically significant. All analyses were made using PC-SAS (version 8.02, SAS Institute, Carey, NC) mixed procedure.

RESULTS

Experiment 1: Effects of 5-HT3 receptor blockade on CCK-induced inhibition of solid food intake

As demonstrated in Fig. 1, there were overall significant main effects of drug treatment on food intake for CCK at 30 min [F(1,88) = 100.58, P<0.0001], 60 min [F(1,88) = 52.36, P<0.0001], and 120 min [F(1,88) = 4.71, P=0.0327], as well as for ondansetron at 30 min [F(1,88) = 10.46, P=0.0017], 60 min [F(1,88) = 12.76, P=0.0006], and 120 min [F(1,88) = 5.12, P=0.0261]. Two-way rmANOVA revealed a significant overall interaction between CCK and ondansetron drug treatments on food intake at 60 min [F(1,88) = 5.01, P=0.0277] and 120 min [F(1,88) = 4.71, P=0.0327]. Systemic
administration of CCK reduced 30, 60, and 120 min chow intake compared to control ($P<0.001$, $P<0.001$, and $P=0.003$, respectively). Ondansetron alone had no significant effect on food intake compared to saline injections ($P=0.61$, $P=0.68$, $P=0.99$; for 30, 60 and 120 min respectively). However, blockade of 5-HT3 receptors by ondansetron attenuated both 30 min ($P=0.013$) and 60 min ($P=0.002$) CCK-induced reduction of food intake, with suppression being completely reversed by 120 min ($P=0.034$).

**Experiment 2: Effects of 5-HT3 receptor blockade on CCK-induced inhibition of sham feeding.**

Two-way rmANOVA revealed an overall significant CCK treatment effect on sham sucrose intake at 30 [$F(1,12) = 47.57, P<0.001$] and 60 min [$F(1,12) = 36.38, P<0.001$]. Sham intake in response to IP administration of CCK was reduced at 30 min ($48.9 \pm 10.4\%$ suppression; $P=0.0032$) and 60 min ($30.9 \pm 8.3\%$ suppression; $P=0.0038$) compared to intake following saline injection. Ondansetron treatment alone did not produce any significant effect on sham intake compared to control ($P=0.87$ and $P=0.84$ for 30 and 60 min, respectively). Blockade of 5-HT3 receptors with ondansetron had no effect on CCK-induced suppression of sham intake ($P<0.05$).

**Experiment 3: Effects of 5-HT3 receptor blockade on CCK-induced suppression of solid food gastric emptying**

Two-way ANOVA revealed a significant treatment effect of CCK [$F(1,15) = 15.55, P=0.0013$], ondansetron [$F(1,15) = 13.98, P=0.002$], and interaction between CCK and ondansetron [$F(1,15) = 19.29, P=0.0005$]. Rats treated with CCK emptied significantly less dry matter ($0.66 \pm 0.14g$) compared with rats given a saline injection ($1.47 \pm 0.07g; P<0.001$) during the one hour test. Ondansetron alone had no effect on the amount of dry matter emptied ($1.41 \pm 0.11g; P=0.88$) from the stomach. As Fig. 3 shows, co-administration of ondansetron with CCK reversed the CCK-induced suppression of gastric emptying of solid chow ($1.45 \pm 0.07g; P<0.001$).

**Experiment 4. Effects of 5-HT3 receptor blockade on CCK-induced suppression of gastric emptying of liquid solutions.**

**0.9% NaCl load**

Two-Way rmANOVA revealed a significant main effect of CCK treatment on 5-min gastric emptying of a 5 ml 0.9% NaCl load [$F(1,108) = 110.61, P<0.0001$], as well as an interaction between CCK and ondansetron [$F(1,108) = 9.9, P=0.0021$]. The percent volume emptied was calculated and results are depicted in Fig. 4. Intraperitoneal administration of ondansetron did not alter 5-min gastric emptying of 0.9% saline ($65.3 \pm 6.0\%$) compared to emptying after saline injection ($70.8 \pm 1.0\%, P=0.63$). CCK administration significantly reduced 5-min gastric emptying ($29.2 \pm 3.7\%$ volume emptied; $P<0.0001$). Co-administration of ondansetron with CCK significantly attenuated CCK-induced reduction of gastric emptying ($42.5 \pm 3.6\%$ volume emptied; $P<0.0054$).

**10% glucose load**

Two-way rmANOVA revealed an overall significant main effect of CCK on gastric emptying of a 10% glucose load [$F(1,32) = 50.53, P<0.0001$], as well as an interaction between CCK and ondansetron [$F(1,32) = 25.56, P<0.0001$]. Intraperitoneal
administration of ondansetron alone had no significant effect on 10 min gastric emptying of a 10% glucose load (41.6 ± 2.8%) compared to saline injection (48.9 ± 1.1%; \( P=0.08 \)). As depicted in Fig. 5, systemically administered CCK significantly reduced gastric emptying (23.9 ± 2.6% volume emptied; \( P<0.0001 \)). However, when ondansetron was co-administered with CCK, gastric emptying increased significantly (37.3 ± 2.9%) compared to the corresponding suppression of gastric emptying induced by CCK (\( P<0.0001 \)).

DISCUSSION

The results of these experiments reveal several important findings. First, we showed that ondansetron, a selective 5-HT3 receptor antagonist, attenuated reduction of food intake by CCK when rats are tested on a solid maintenance diet. This extends previous reports indicating that inhibition of food intake by CCK is mediated via 5-HT3 receptors when rats were tested on a preferred 15% sucrose solution (11, 22). Second, systemic administration of ondansetron did not alter CCK-induced suppression of sham feeding, suggesting that 5-HT3 receptors mediate CCK-induced satiation through indirect mechanisms involving gastric/post-gastric feedback. Third, blockade of 5-HT3 receptors attenuated inhibition of gastric emptying by CCK of both solid and liquid loads. This indicates that 5-HT3 receptors participate in CCK-induced satiation through mechanisms involving gastric emptying.

The sham feeding preparation allows ingested nutrients to drain freely from the stomach and results in an increase in food intake due to the absence of gastric and post-gastric negative feedback inhibition on feeding. CCK has been shown to directly induce satiety in the sham feeding preparation (16, 20) in the absence of several possible additive endogenous satiety mechanisms, by directly activating receptors located on terminals of sensory vagal afferent fibers (35, 51). Thus, the use of this preparation allowed us to dissect the potential direct or indirect participation of 5-HT3 receptors in mediating CCK-induced satiation. The current results demonstrate that ondansetron reduced the satiety actions of CCK in real-feeding experiments, but did not affect the ability of CCK to inhibit sham feeding. Thus, ondansetron appears to block the anorectic actions of CCK when it is acting in conjunction with other post-oropharyngeal feedback signals, but is unable to directly affect the satiating properties of CCK alone.

Inhibition of gastric emptying by CCK is one mechanism involved in the satiety action of CCK (44). In addition, intraintestinal nutrient infusion into the upper small intestine, reduces food intake and gastric emptying (9, 10, 18, 26, 34, 41, 54). The nutrient-induced reduction of food intake and gastric emptying has been shown to be mediated by both CCK-A and 5-HT3 receptors (10, 49, 53, 59). Thus, 5-HT3 receptors may mediate CCK-induced reduction of food intake by interfering with CCK’s ability to inhibit gastric emptying. Indeed, our results demonstrated that blockade of 5-HT3 receptors caused an attenuation of CCK-induced inhibition of gastric emptying. This occurred when animals were allowed to freely consume a solid maintenance diet as well as when the stomach was filled with a liquid load. In addition to the chemical and colligative characteristics of nutrients that affect gastric emptying, other properties such as osmolality and caloric density have also been shown to inhibit gastric emptying (2, 18, 50). Moran and colleagues (42) have demonstrated that endogenous CCK is involved in
inhibition of gastric emptying by intragastric glucose and maltose. In our studies, ondansetron attenuated CCK-induced inhibition of gastric emptying of both isotonic saline, as well as 10% glucose, indicating that 5-HT3 receptors mediate inhibition of gastric emptying by CCK independent of nutritive and osmotic feedback signals.

The effects of ondansetron on gastric emptying have been examined previously under a number of different experimental conditions, with conflicting outcomes. Some studies using acute systemic administration of ondansetron have shown an inhibition of gastric emptying (17), while others showed an increase in gastric emptying (27, 39). A number of factors may account for this discrepancy. For example, Miyata et. al (39) and Ito et al. (27) examined gastric emptying of glass powder/beads and found that systemic administration of ondansetron at low doses (0.01 – 0.1mg/kg) increased gastric emptying. On the other hand, Forster and Dockray (17) have shown that peripheral administration of similar low doses of ondansetron enhanced peptone-induced suppression of gastric emptying. In contrast, our results showed that ondansetron alone, at the dose we tested (1.0 mg/kg), had no effect on gastric emptying of a complete solid meal, glucose, or isosmotic non-nutritive load. One might argue that the dose of ondansetron used in our studies may have a subthreshold effect whereby ondansetron may not be able to increase gastric emptying beyond the control condition. However, in our experiments following saline injection, neither saline nor glucose load emptied completely during the testing period (i.e. not all 5ml emptied).

More recently Raybould and colleagues (49) have shown that inhibition of gastric emptying by intraduodenal glucose solution was attenuated in response to blockade of 5-HT3 receptors. Several reasons may account for the differences in their results compared to ours. While the concentrations of the solution infused were comparable between the studies (10% vs. 8.9% glucose), the discrepancy between our results and theirs may be explained by the large difference in both the volume of glucose infused and the site of infusion. For example, Raybould et. al infused a total volume of 0.56 ml directly into the duodenum whereas we loaded a total volume of 5.0 ml directly into the stomach. Therefore, failure of ondansetron to enhance emptying in our studies could be due to a difference in total amount of glucose coming in contact with the intestinal lumen. Suppression of food intake by intestinally perfused nutrients as well as the rate of gastric emptying has been shown to depend on length of the gut contacted (31, 37, 38). Furthermore, while in our studies we used ondansetron, that acts selectively at the 5-HT3 receptors (27), Raybould et al., used tropisetron, that has broader serotonergic activity binding to both 5-HT3 and 5-HT4 receptors (13, 14, 27); the latter being shown to participate in the control of gastric motility (52, 57). It is clear that further studies are needed to determine the mechanisms by which 5-HT3 receptors mediate glucose-induced inhibition of gastric emptying. It is worth noting however that the attenuating effect of ondansetron on CCK-induced inhibition of solid gastric emptying is complete while its effect on CCK-induced inhibition of liquid gastric emptying is partial. It is known that solid matter empties at a slower rate from the stomach than does a liquid load. Furthermore, the relative gastric load was undoubtedly greater in the solid gastric emptying experiment, where rats’ intake of chow and water was unrestricted for one hour compared to the 5 ml liquid gastric load in experiment 4. This is evident by the presence
of a substantial amount of solid matter within the stomach at one hour. In contrast, over half of the gastric liquid load had emptied in 10 min. Thus, in the solid gastric emptying experiment there was most likely a prolonged and enhanced gastric distension, probably resulting in greater release of 5-HT (32), ultimately enhancing the activity of 5-HT3 receptors. The fact that ondansetron alone did not enhance gastric emptying may suggest that the effects of ondansetron on the inhibition of gastric emptying by CCK is CCK dependent.

Our data show that 5-HT3 receptor mediation of CCK-induced suppression of real feeding involves gastric/post-gastric feedback. If CCK had a direct action on 5-HT3 receptors, then ondansetron should have attenuated CCK-induced suppression of sham feeding. CCK’s inhibition of both feeding as well as gastric emptying has been shown to be dependent upon intact vagal afferent pathways (54). Likewise, 5-HT3 receptor participation in the control of gastric emptying has also been demonstrated to be mediated along capsaicin-sensitive extrinsic afferent nerve fibers (4). In view of the fact that CCK and 5-HT activate distinct separate populations of vagal afferent fibers (23), the present findings suggest that CCK-induced suppression of sham feeding is mediated along vagal afferent fibers not directly originating from 5-HT3 receptors.

An additional explanation of our findings could be that CCK acts as a secretagogue for the release of 5-HT, which, in turn activates 5-HT3 receptors. This could result in an enhanced anorectic effect, if one were to imagine that CCK would promote enterochromaffin cell release of 5-HT, which then promotes enteroendocrine “T” cell release of CCK (48). Considering that both serotonergic and cholecystokininergic systems elicit an anorectic response that includes a delay in gastric emptying, it would make sense to believe that cooperation of these two systems within the periphery would allow for a greater control of feeding behavior and related gastric functions.

The serotonergic system, like the cholecystokininergic system, has been investigated extensively for its incretin-like involvement in the control of food intake. Moreover, investigations of the serotonergic system have attempted to determine the percent contribution of 5-HT in food intake and whether 5-HT-induced suppression of food intake is directly or indirectly mediated. Original exploration of 5-HT’s effect on sham feeding indicated that peripheral 5-HT inhibits sham intake (45, 46) in the absence of a complete satiety sequence (56). Neill and Cooper (45) demonstrated that administration of a peripheral acting 5-HT2a/2c receptor antagonist xylamidine, attenuated systemically administered 5-HT-induced inhibition of sham feeding. However, to date there has been no direct study confirming 5-HT3 receptor involvement in 5-HT-induced suppression of sham intake using a selective 5-HT3 receptor antagonist. Thus, it is still unknown whether 5-HT3 receptors directly mediate 5-HT induced anorexia or whether their participation involves gastric/post-gastric feedback similar to their mediation of CCK-induced satiety.

Our findings indicate that CCK’s actions are mediated by 5-HT3 receptors through indirect mechanisms involving gastric distension and gastric emptying. A recent report by Mazda and colleagues (32) showed that gastric distension promotes 5-HT release, which induces c-fos expression in the dorsal medulla via 5-HT3 receptor activation. Since CCK-induced inhibition of gastric emptying results in an increase in gastric distension (4), this would promote 5-HT release, acting on 5-HT3 receptors to further inhibit gastric emptying. Thus, blockade of 5-HT3 receptors would most likely
attenuate CCK-induced reduction in gastric emptying by limiting 5-HT3 receptor mediated inhibition arising from gastric distension-induced release of 5-HT. Consequently, ondansetron would only be able to attenuate CCK-induced suppression of intake and gastric emptying when gastric distension is not impeded. The sham feeding results support this notion, whereby ondansetron was unable to attenuate CCK-induced satiation in the absence of gastric distension.

In conclusion, the present findings demonstrate that 5-HT3 receptors mediate CCK-induced satiation through indirect mechanisms as part of a feedback cascade involving inhibition of gastric emptying. Additionally, we have provided evidence that in the absence of gastric and post-gastric feedback signals, 5-HT3 receptors do not mediate CCK-induced satiation. We propose that CCK-induced suppression of gastric emptying most likely invokes an increase in gastric distension, resulting in elevated 5-HT release which acts on peripheral 5-HT3 receptors to aid in the overall satiating signal. Taken with our previous findings, these results suggest that 5-HT3 receptors mediate CCK-induced satiation through indirect mechanisms dependent upon gastric and/or post-gastric feedback.
ACKNOWLEDGEMENTS

The authors wish to thank Melissa Carelle, Bart De Jonghe, David M. Savastano, and Jonathan G. Stine for their help with these studies.
Figure Legends

Fig. 1. Administration of CCK (1.0 µg/kg, IP) significantly reduced rat chow intake at all time points, compared to control in food deprived rats. Co-administration of ondansetron (Ond; 1.0 mg/kg, IP) and CCK significantly attenuated CCK-induced reduction of 30, 60, and 120 min rat chow intake.* = $P < 0.05$ from saline at given time. † = $P < 0.05$ from corresponding CCK at given time.

Fig. 2. Administration of CCK (2.0 µg/kg, IP) significantly reduced sucrose intake compared to control sham-feeding rats. Ondansetron (Ond; 1.0 mg/kg, IP) alone did not alter 30 or 60 min 15% sucrose intake compared to control. Ondansetron, when co-administered with CCK was unable to attenuate CCK-induced reduction of 30 or 60 min sucrose intake. * = $P < 0.05$ from saline.

Fig. 3. Administration of CCK (1.0 µg/kg, IP) significantly reduced the amount of dry matter emptied from the stomach at 60 min, compared to control in food deprived rats. Co-administration of ondansetron (Ond; 1.0 mg/kg, IP) and CCK significantly attenuated CCK-induced suppression of gastric emptying of dry chow. * = $P < 0.001$ from saline. † = $P < 0.001$ from corresponding CCK suppression.

Fig. 4. Intraperitoneal administration of CCK (1.0µg/kg) significantly suppressed 5-min gastric emptying of 0.9% saline compared to control in food deprived rats. Ondansetron (Ond; 1.0mg/kg, IP) did not alter 5-min saline emptying compared to emptying after IP saline administration. Co-administration of ondansetron with CCK significantly attenuated CCK-induced reduction of 5-min gastric emptying. * = $P < 0.01$ from saline. † = $P < 0.01$ from corresponding CCK suppression.

Fig. 5. Intraperitoneal administration of CCK (1.0µg/kg) significantly suppressed 10 min gastric emptying of 10% glucose compared to control in food deprived rats. Ondansetron (OND; 1.0mg/kg, IP) did not alter 10 min glucose emptying compared to emptying after IP saline administration. Co-administration of CCK with ondansetron significantly attenuated CCK-induced reduction of 10 min gastric emptying of a 10% glucose solution. * = $P < 0.001$ from saline. † = $P < 0.001$ from corresponding CCK suppression.
Figure 1

- Saline
- Ond 1mg/kg
- CCK 1µg/kg
- CCK 1µg/kg + Ond 1mg/kg

Chow Intake (g)

Time (min)
Figure 2

- Ond 1mg/kg
- CCK 2µg/kg
- CCK 2µg/kg + Ond 1mg/kg

Saline = 100%

Percent of Control

Time (min)

0 10 20 30 40 50 60 70 80 90 100 110 120

* * *
Figure 3

Dry Matter Gastric Emptying (grams emptied)

60 min

Saline
Ond 1mg/kg
CCK 1µg/kg
CCK 1µg/kg + Ond 1mg/kg

* †
Figure 4

5-min Gastric Emptying of 0.9% NaCl

- Saline
- Ond 1mg/kg
- CCK 1μg/kg
- CCK 1μg/kg + Ond 1mg/kg

* p < 0.05 compared to Saline
† p < 0.05 compared to CCK 1μg/kg + Ond 1mg/kg
Figure 5

10 min Gastric Emptying of 10% Glucose

Saline
OND 1mg/kg
CCK 1µg/kg
CCK 1µg/kg + OND 1mg/kg

Percent Volume Emptied
REFERENCES:


7. **Cooper SJ and Dourish CT.** Multiple cholecystokinin (CCK) receptors and CCK-monoamine interactions are instrumental in the control of feeding. *Physiol Behav* 48: 849-857, 1990.


