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, Pennsylvania 16802-6504

Submitted 4 May 2004; accepted in final form 2 June 2004

Hayes, Matthew R., Rachael L. Moore, Samit M. Shah, and Mihai Covasa. 5-HT₃ receptors participate in CCK-induced suppression of food intake by delaying gastric emptying. Am J Physiol Regul Integr Comp Physiol 287: R817–R823, 2004. First published June 10, 2004; 10.1152/ajpregu.00295.2004.—Serotonin type 3 (5-HT₃) receptors have been shown to participate in the negative-feedback control of food intake. We 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 they are 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. Intrapерitoneal 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. Because CCK-induced inhibition of sham feeding does not depend on additive gastric/postgastric-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.

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 (for review see Ref. 25). Systemic serotonergic activity has been shown to induce an anorectic response through activation of 5-HT₃ receptors under a number of paradigms (1, 21). One such response is the mediation of nutrient-induced inhibition of gastric emptying (49). Similar to 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). Although 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 CCKₐ receptors cooperate independently 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 underlying physiological mechanisms are unclear. If we consider 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) previously reported that 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 the 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. Because CCK-induced inhibition of sham feeding does not depend on gastric/postgastric-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 caloric and noncaloric 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 nonnutritive liquid, gastric loads, a

Address for reprint requests and other correspondence: M. R. Hayes, Dept. of Nutritional Sciences, College of Health and Human Development, The Pennsylvania State Univ., 126 South Henderson, University Park, PA 16802.
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. The rats were maintained on a 12:12-h light-dark cycle (lights off at 1800) and were habituated to laboratory conditions for 1 wk before surgery or initiation of experiments. The protocols were approved by The Pennsylvania State University Institutional Animal Care and Use Committee. The drugs used in these experiments were cholecystokinin-octapeptide sulfate (American Peptide, Sunnyvale, CA) and ondansetron (2.0 mg/ml; Burns Veterinary Supply, Rockville, MD), a selective 5-HT3 receptor antagonist. All drugs were dissolved in sterile 0.9% saline and administered via intraperitoneal injection in a volume of 1.0 ml/kg body wt, with all rats receiving the same drug treatment on the same day. The CCK doses (1.0 and 2.0 μg/kg) were chosen on the basis of numerous CCK dose-response studies demonstrating reliable and replicable suppression of food intake as well as gastric emptying (9, 22). For sham feeding, CCK was administered at a dose of 2.0 μg/kg to produce a percent suppression in sham feeding comparable to that seen in real feeding by CCK. The ondansetron dose of 1.0 mg/kg has been used previously in experiments exploring 5-HT3 receptor mediation of CCK’s effects (11, 22).

Surgical Procedures

Gastric cannulation. In rats used in sham-feeding studies, stainless steel gastric cannulas were implanted 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 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 leakage, 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 (17 h) rats (230–260 g) were injected intraperitoneally with NaCl, ondansetron (1.0 mg/kg), CCK (1 μg/kg), or CCK (1 μg/kg) + ondansetron (1.0 mg/kg). At 5 min after the injection, a preweighed amount of standard rodent chow was presented to the rats, and food intake was recorded at 0.5, 1, and 2 h after injection, with spillage that was collected in a tray placed below the cage taken into account. Each drug treatment was separated by ≥48 h and was bracketed by an 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, each of five rats was equipped with a chronic gastric cannula and sham fed a 15% (wt/vol) sucrose solution. After 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 (37°C) tap water. 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 NaCl, ondansetron (1.0 mg/kg), CCK (2.0 μg/kg), or CCK (2.0 μg/kg) + ondansetron (1.0 mg/kg). Immediately after injection, rats were presented with a calibrated glass burette filled with 15% (wt/vol) sucrose solution, and intake was recorded every 5 min for the ensuing 60 min. Each drug treatment was separated by ≥48 h and was bracketed by an NaCl condition.

Experiment 3: Effects of 5-HT3 Receptor Blockade on CCK-Induced Suppression of Gastric Emptying of Solid Food

In a separate group of 19 rats (260–450 g), we examined gastric emptying of maintenance rat chow. After an overnight fast, rats were injected intraperitoneally with NaCl, ondansetron (1.0 mg/kg), CCK (1 μg/kg), or CCK (1 μg/kg) + ondansetron (1.0 mg/kg). Preweighed rat chow was presented 5 min later. All rats were euthanized by CO2 asphyxiation 60 min after chow presentation. For measurement of 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. For calculation of the amount of the test meal that emptied from the stomach, the remaining stomach contents were dried overnight at 100°C and compared with the original moisture content of the chow ingested, determined by drying preweighed 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) × 100%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) was adapted to experimental conditions for 1 wk before testing. After an overnight fast, water was removed 1 h before drug administration. At 0900, rats received an intraperitoneal injection containing NaCl, ondansetron (1.0 mg/kg), CCK (1.0 μg/kg), or ondansetron (1.0 mg/kg) + CCK (1.0 μg/kg). At 5 min after drug administration, 5 ml of 0.9% NaCl or 10% glucose solution (wt/vol) containing 0.006% phenol red were instilled into the rat’s stomach via an orally inserted 8-Fr polyethylene intragastric tube. Rats were immediately returned to their home cage after gastric load. After a 5-min (NaCl) or a 10-min (glucose) 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 phenol indicator was no longer visible. 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 550 nm. Briefly, a 1.0-ml sample from the centrifuged gastric contents was buffered with 24.5 ml 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 ≥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 described in detail previously (29, 55).

Statistical Analyses

Data for each respective study were analyzed separately and expressed as means ± SE. Rat chow and 15% sucrose solution intakes

AJP-Regul Integr Comp Physiol • VOL 287 • OCTOBER 2004 • www.ajpregu.org
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 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, Cary, NC) mixed procedure.

RESULTS

Experiment 1: Effects of 5-HT\(_3\) 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 repeated-measures ANOVA 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 with control \((P < 0.001, P < 0.001, \text{and } P = 0.003, \text{respectively})\). Ondansetron alone had no significant effect on food intake compared with saline injections \((P = 0.61, P = 0.68, \text{and } P = 0.99 \text{for 30, 60, and 120 min, respectively})\). However, blockade of 5-HT\(_3\) receptors by ondansetron attenuated 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-HT\(_3\) Receptor Blockade on CCK-Induced Inhibition of Sham Feeding

Two-way repeated-measures ANOVA revealed an overall significant CCK treatment effect on sham sucrose intake at 30 min \([F(1,12) = 47.57, P < 0.001]\) and 60 min \([F(1,12) = 36.38, P < 0.001, \text{Fig. 2}]\). Sham intake in response to intra-peritoneal administration of CCK was reduced at 30 min \((48.9 \pm 10.4\% \text{ suppression, } P = 0.0032)\) and 60 min \((30.9 \pm 8.3\% \text{ suppression, } P = 0.0038)\) compared with intake after saline injection. Ondansetron treatment alone did not produce any significant effect on sham intake compared with control \((P = 0.87 \text{ and } P = 0.84 \text{ for 30 and 60 min, respectively})\). Blockade of 5-HT\(_3\) receptors with ondansetron had no effect on CCK-induced suppression of sham intake \((P < 0.05)\).

Experiment 3: Effects of 5-HT\(_3\) Receptor Blockade on CCK-Induced Suppression of Gastric Emptying Solid Food

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

Experiment 4: Effects of 5-HT\(_3\) Receptor Blockade on CCK-Induced Suppression of Gastric Emptying of Liquid Solutions

0.9% NaCl load. Two-way repeated-measures ANOVA 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 ± 6.0%) compared with emptying after saline injection (70.8 ± 1.0%, \( P = 0.63 \)). CCK administration significantly reduced 5-min gastric emptying (29.2 ± 3.7% volume emptied, \( P < 0.0001 \)). Ondansetron + CCK significantly attenuated CCK-induced reduction of gastric emptying (42.5 ± 3.6% volume emptied, \( P < 0.0001 \)).

**10% glucose load.** Two-way repeated-measures ANOVA 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 with saline injection (48.9 ± 1.1%, \( P = 0.08 \)). Systemically administered CCK significantly reduced gastric emptying (23.9 ± 2.6% volume emptied, \( P < 0.0001 \); Fig. 5). However, when ondansetron + CCK was administered, gastric emptying increased significantly (37.3 ± 2.9%) compared with 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-HT\(_3\) 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-HT\(_3\) 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-HT\(_3\) receptors mediate CCK-induced satiation through indirect mechanisms involving gastric/postgastric feedback. Third, blockade of 5-HT\(_3\) receptors attenuated inhibition of gastric emptying by CCK of solid and liquid loads. This indicates that 5-HT\(_3\) 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 postgastric 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 present 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 postoropharyngeal 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 CCKₐ 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) 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 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 showed an inhibition of gastric emptying (17), whereas 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 (0.01–0.1 mg/kg) increased gastric emptying. On the other hand, Forster and Dockray (17) showed 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 nonnutritive 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 after saline injection, neither saline nor glucose load emptied completely during the testing period (i.e., not all 5 ml emptied).

More recently, Raybould and colleagues (49) showed 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 with ours. Although 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 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, although in our studies we used ondansetron, which acts selectively at the 5-HT₃ receptors (27), Raybould et al. used tropisetron, which has broader serotonergic activity binding to 5-HT₃ and 5-HT₁ 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. However, the attenuating effect of ondansetron on CCK-induced inhibition of solid gastric emptying is complete, whereas 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 1 h compared with 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 1 h. 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 are CCK dependent.

Our data show that 5-HT₃ receptor mediation of CCK-induced suppression of real feeding involves gastric/postgastric 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 feeding as well as gastric emptying has been shown to be dependent on intact vagal afferent pathways (54). Similarly, 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 “I” cell release of CCK (48). If we were to consider that 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, similar to 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 to 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-HT3 receptor antagonist, xylamidine, attenuated systemically administered 5-HT-induced inhibition of sham feeding. However, 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 unknown whether 5-HT3 receptors directly mediate 5-HT-induced anorexia or whether their participation involves gastric/postgastric 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. Because 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 be able to attenuate CCK-induced suppression of intake and gastric emptying only 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 postgastric-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 satiety signal. Taken with our previous findings, these results suggest that 5-HT3 receptors mediate CCK-induced satiation through indirect mechanisms dependent on gastric and/or postgastric feedback.

ACKNOWLEDGMENTS

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

REFERENCES


Ladenheim EE, Wohn A, White WO, Schwartz GJ, and Moran TH. **5-HT3 RECEPTORS AND CCK-INDUCED DELAY IN GASTRIC EMPTYING**


