Serotonin type-3 receptors mediate cholecystokinin-induced satiation through gastric distension

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Hayes, Matthew R., Fiona M. Chory, Claire A. Gallagher, and Mihai Covasa. Serotonin type-3 receptors mediate cholecystokinin-induced satiation through gastric distension. Am J Physiol Regul Integr Comp Physiol 291: R115–R123, 2006. First published February 16, 2006; doi:10.1152/ajpregu.00002.2006.—We have previously shown that serotonin type-3 (5-HT3) receptors mediate cholecystokinin (CCK)-induced satiation and that this effect is dependent on postpharyngeal feedback. However, the independent contributions of gastric and intestinal feedback in 5-HT3 receptor mediation of suppression of food intake by CCK have not been determined. Using a sham-feeding preparation combined with intraduodenal sucrose infusion, we show that blockade of 5-HT3 receptors by ondansetron (1 mg/kg ip) had no effect on suppression of sham feeding by intraduodenal 15% sucrose infusion (4 ml/10 min), CCK (2 μg/kg ip) administration, or the combination of the two treatments. In separate experiments consisting of either sham-feeding rats that received gastric distension with the use of a balloon or real-feeding rats whose stomachs were distended using gastric loads of saline after the occlusion of the pylorus, we tested the hypothesis that gastric feedback signals are necessary for activation of 5-HT3 receptors. Ondansetron significantly attenuated suppression of sham sucrase intake after a 10-ml gastric balloon distension (30.5 ± 2.2 vs. 20.2 ± 2.2 ml, respectively) and gastric distension combined with CCK (21.9 ± 1.4 vs. 12.0 ± 1.7 ml, respectively). When intestinal feedback was eliminated in a real-feeding paradigm by closing the pylorus using a cuff preparation, ondansetron attenuated suppression of sucrose intake produced by a 10-ml saline gastric load (6.8 ± 0.7 vs. 4.2 ± 0.4 ml, respectively). Finally, when CCK (1 μg/kg) was administered in combination with a 5-ml saline gastric load in a real-feeding preparation, ondansetron significantly attenuated suppression of sucrose intake by CCK (9.0 ± 0.9 vs. 6.3 ± 0.5 ml, respectively), as well as the enhanced suppression of intake by CCK plus gastric load (6.9 ± 0.6 vs. 4.6 ± 0.5 ml, respectively). These findings demonstrate that CCK-induced activation of 5-HT3 receptors requires gastric, but not intestinal feedback.

WITHIN-MEAL CONTROL OF FOOD intake has been postulated to occur in response to neuronal integration of stomach and intestinal feedback signals (42, 46, 52). Anorectic signals originating from the stomach occur almost exclusively in response to volumetric distension of the organ (41, 42), whereas intestinal anorectic signals originate largely in response to the nutrient and chemical properties of a meal (17, 46, 60). A considerable amount of work has examined the mechanisms by which the presence of nutrients within the small intestine results in satiation for food. The majority of this evidence agrees that intraintestinal nutrients reduce food intake largely through vagal afferent signaling resulting from neuronal activation via paracrine and/or endocrine products of intestinal origin (for a review see Ref. 46). In addition to directly signaling for satiation, these intestinally derived gut peptides and neurotransmitters stimulate vagovagal reflexes and reduce the rate of gastric emptying, which ultimately results in retention of stomach contents (34, 36, 37). Although it is known that gastrointestinal signals of satiation are primarily mediated along the gastric and hepatic branches of the vagus (40), as well as splanchnic afferents (54), little work has been done to identify the mechanisms and neuronal substrates responsible for mediating these signals.

Whereas the neuronal substrate(s) responsible for mediating gastric satiation signals by and large have remained unidentified, considerable attention has been devoted to the intestinally derived satiety signal cholecystokinin (CCK) for both its ability to inhibit gastric emptying (21, 35, 37) and to potentiate gastric distension satiation signaling (26, 37, 51). However, CCK-induced enhancement of satiation signaling is not limited exclusively to its interaction with gastric distension. In fact, CCK-induced activation of CCK-1 receptors has been shown to enhance both suppression of food intake and neuronal signaling when simultaneously combined with other anorectic signals including, but not limited to intraduodenal nutrients (9, 59), amylin (2, 31), leptin (1, 32), bombesin (57), and serotonin (5-HT) (6, 7, 19). Despite the fact that activation of CCK-1 receptors is critical for the aforementioned interactions in control of food intake, CCK-1 receptors themselves do not often directly mediate noncholecystokininergic neuronal signaling, such as those derived from gastric distension (55, 57). Therefore, the interaction between CCK and gastric distension in suppression of food intake likely involves participation of other satiating neuronal signals. 5-HT is one likely candidate, because it is released in response to gastric distension (33) and synergistically suppresses food intake when systemically combined with CCK (19). However, participation of the serotonergic system in mediating gastric distension-induced suppression of food intake has not been tested.

CCK and 5-HT are released in close proximity from gastrointestinal endocrine cells in response to various stimuli, such as gastric distension (5-HT; Ref. 33) and the presence of nutrients within the small intestine (both CCK and 5-HT; Refs. 29, 46, 47). The physiological actions of CCK, including satiation (46, 47) and suppression of gastric emptying (45), are largely mediated by vagal sensory nerve fibers. Likewise, activation of
vagal sensory nerve fibers occurs when 5-HT is endogenously released in response to gastrointestinal stimulation (33, 44). Whereas cholecystokininergic or serotonergic excitation of peripheral sensory nerve fibers independently reduces meal size, reports have also shown that these two systems work together in control of food intake and various neural modulatory functions (6, 19, 30). Indeed, our laboratory has recently shown that simultaneous systemic administration of CCK and 5-HT synergistically suppress intake (19), further supporting the notion of cooperation between CCK and 5-HT in the overall control of food intake.

Of the seven distinct classes of 5-HT receptor types identified, a number of these have been implicated in mediation of CCK-induced satiation. Specifically, we (21, 22) and others (11) have previously shown that administration of ondansetron, a selective 5-HT type-3 (5-HT3) receptor antagonist, attenuates suppression of intake resulting from systemic CCK administration. Likewise, 5-HT3 receptors have been shown to mediate both intestinal nutrient- and CCK-induced inhibition of gastric emptying (21, 44). Recently, however, we reported that ondansetron was unable to alter CCK-induced suppression of sham feeding (21). This finding indicates that gastric/postgastric feedback is necessary for 5-HT3 receptor mediation of CCK-induced satiation; however, the underlying physiological mechanisms are still unclear.

Previous reports have shown that when CCK is administered in combination with gastric distension, an enhanced suppression of food intake is observed (26, 37, 51). Likewise, CCK and gastric distension have been shown to mutually amplify stimulation of vagal afferent fibers (50), as well as enhance neuronal activation of the dorsal hindbrain (55). Whereas these previous findings show CCK-induced retention of stomach contents enhances CCK’s satiating signal via gastric distension, the specific neuronal substrates responsible for mediating gastric distension’s satiating signals have not been completely elucidated. Mazda et al. (33) suggested that gastric distension induces activation of 5-HT3 receptors by releasing endogenous 5-HT. Therefore, we hypothesize that the enhancement of CCK-induced satiation by gastric distension involves 5-HT3 receptor activation. This is supported by our previous findings showing that in the absence of postoropharyngeal feedback, 5-HT3 receptors do not mediate CCK-induced satiation (21).

No studies, however, have addressed the specific contributions of gastric vs. postgastric feedback participation in this effect. Therefore, by using a sham-feeding preparation combined with intestinal nutrient infusion we first tested the hypothesis that gastric feedback signals are necessary for participation of 5-HT3 receptors in suppression of food intake by CCK. Blockade of 5-HT3 receptors had no effect on suppression of sham feeding by CCK, nutrient infusion, or CCK plus nutrient infusion combination. In the next study, we occluded the pylorus by using an inflatable cuff and examined the independent contribution of 5-HT3 receptors in mediating suppression of intake by gastric distension in a real-feeding paradigm and in the absence of intestinal feedback. This preparation enabled us to examine 5-HT3 receptor mediation of gastric distension-induced satiation when the volume of distension and the rate of stomach fill were either controlled by the rate and duration of ingestion or were experimentally manipulated via gavaged gastric loads. We report that 5-HT3 receptors mediate suppression of food intake by gastric distension in the absence of intestinal negative feedback.

To assess participation of 5-HT3 receptors in suppression of food intake by gastric distension when combined with CCK, in the next two studies, we measured sham and real feeding of ondansetron-pretreated rats after stomach distension via balloon inflation or gastric preloads. Blockade of 5-HT3 receptors attenuated suppression of sham feeding by balloon distension and by CCK combined with balloon distension. Furthermore, we found that ondansetron treatment attenuated suppression of real feeding after concomitant administration of CCK and gastric preloads.

**MATERIALS AND METHODS**

**Animals and Drugs**

Adult (250–350 g) male Sprague-Dawley rats (Harlan, Indianapolis, IN) were individually housed (wire hanging cages) in a temperature- and light-controlled environment with a 12:12-h light-dark cycle (lights off at 1800). Rats were provided ad libitum access to standard rat chow (Purina 5001) and water, except as indicated in the experimental procedure when they were deprived of food but not water overnight (16 h). Before testing, animals were adapted to experimental conditions for 1 wk. This protocol was approved by The Pennsylvania State University Institutional Animal Care and Use Committee.

The drugs used in these experiments were CCK octapeptide sulfate (CCK-8; American Peptide, Sunnyvale, CA) and ondansetron HCl (a gift from GlaxoSmithKline, Barnard Castle, UK), a selective 5-HT3 receptor antagonist. All drugs were dissolved in sterile 0.9% saline and were administered via an intraperitoneal injection in a volume of 1.0 ml/kg body wt. 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 both real and sham feeding (8, 21, 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 as previously described (21). On the basis of our laboratory’s previous dose-response experiments examining 5-HT3 receptor mediation of intestinal nutrient- (48) and CCK-induced (19, 22) satiation, the dose of 1.0 mg/kg of ondansetron was chosen in the current studies because of its ability to effectively attenuate suppression of real feeding by these two stimuli, with higher doses producing no greater effect (19, 48).

**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 (60). 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.

**Intestinal cannulation.** Rats used in intraintestinal infusion studies were also fitted with chronic duodenal catheters while anesthetized for the purpose of drug delivery. A duodenal cannula, consisting of a 22-cm length of silicone rubber tubing (0.025 in. ID, 0.047 in. OD; Dow Corning, Midland, MI), was inserted 2 cm distal to the pylorus and advanced 6 cm within the duodenal lumen in an aboral direction. The
The rats were allowed to recover from surgery for 7 days before tunneled subcutaneously to emerge at the dorsal aspect of the neck. Its integrity and proper positioning. Finally, the Silastic tubing was the pylorus. The cuff was then inflated with 0.6 ml of water to verify inflatable half of the cuff was positioned along the ventral aspect of the pylorus. The cuff was then inflated with 0.6 ml of water to verify its integrity and proper positioning. Finally, the Silastic tubing was inserted through an incision in the muscles of the abdominal wall and tunneled subcutaneously to emerge at the dorsal aspect of the neck. The rats were allowed to recover from surgery for 7 days before testing began.

After completion of the experiment, the effectiveness of each cuff was assessed by measuring blood glucose levels after an oral glucose tolerance test (52) with the cuff inflated and deflated as described in our previously published work (10). If blood glucose values for a given rat were the same or higher when the cuff was inflated than when it was deflated, we assumed that the glucose solution had escaped through the inflated cuff into the duodenum, and data obtained from that animal were discarded. In addition, cuff placement and patency was also determined in a postmortem examination as previously described (10). Each rat’s cuff was inflated with 0.6 ml of water, followed by a 10-ml gavage of a dye solution. Each rat was then immediately euthanized via CO2 asphyxiation, and its stomach, pyloric cuff, and proximal duodenum were exteriorized via midline celiotomy. A hemostat was applied to the cardiac portion of the stomach. A 2-cm segment of the duodenum, immediately distal to the cuff, was resected, opened, and blotted (mucosa side down) on a piece of filter paper. If dye was visible on the filter paper after the blotting, data for that animal were discarded.

**Experiment 1: 5-HT3 Receptor Blockade on Inhibition of Sham Feeding by CCK Combined with Intestinal Sucrose Infusion**

After recovery from surgery, rats (n = 8) equipped with chronic gastric and intestinal cannulas were trained to sham feed a 15% (wt/vol) sucrose solution after an overnight fast. During both training and testing days at 0900, 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 (60). Training was considered complete when sham baseline intakes were consistent and did not vary more than 8–10%, which was achieved after ~4–5 sucrose exposures. During testing, intraperitoneal drug administration consisted of either NaCl, ondansetron (1.0 mg/kg), CCK (2.0 μg/kg), or a combined injection of CCK (2.0 μg/kg) and ondansetron (1.0 mg/kg). Five minutes after injection, intestinal infusion of either 0.9% NaCl or 15% sucrose solution begun at a rate of 0.4 ml/min for 10 min. This infusion rate has been shown to be within the physiological range of gastric emptying (24). Simultaneous with the start of intestinal infusion, rats were presented with a calibrated glass burette filled with 15% sucrose solution and sham intake was recorded every 5 min for the ensuing 30 min. Each injection/infusion treatment was separated by a minimum of 48 h and was bracketed by a control NaCl/NaCl condition. A minimum of two repetitions for each injection/infusion combination was conducted for each experiment.

**Experiment 2: 5-HT3 Receptor Mediation of Gastric Distension-Induced Suppression of Real Feeding After Occlusion of the Pylorus**

Beginning on postoperative day 2, rats (n = 10) implanted with pyloric cuffs were habituated to cuff manipulations by inflating the cuff with increasing volumes of water (up to 0.6 ml), as well as receiving gavage loads. In addition, rats were familiarized to 15% sucrose solution for 7 days, beginning 1 wk after surgery and ending just before the start of experimental testing. Sucrose recruitment consisted of an overnight fast (16 h) followed at 0900 by cuff manipulation, sham gavage load, and subsequent presentation of 15% sucrose solution for 30 min. For both sucrose training and experimental testing, rats were tested every other day, including weekends. On nontesting days, the rats were handled and pyloric cuffs were inflated and deflated so that the rats would be less likely to associate sensations resulting from cuff manipulations with the presentation of food.

On test days at 0900, overnight-food-deprived (16 h) rats received an intraperitoneal injection of either saline or ondansetron (1.0 mg/kg) with their cuffs either open (deflated) or closed (inflated). Five minutes after drug administration, rats received either a 5- or 10-ml load of 0.9% (wt/vol) NaCl instilled into the stomach with a syringe by hand over a period of 1 min via an orally inserted 8-Fr polyethylene intragastric tube, or the inserted tube without gastric load (sham load). Immediately after gastric/sham load, rats were returned to their home cage and were presented with a calibrated glass burette filled with 15% sucrose (wt/vol) solution. Intakes were recorded to the nearest 0.1 ml at baseline and every 5 min for 30 min. Each drug/cuff/gastric load treatment was separated by a minimum of 48 h and bracketed by a control condition (NaCl injection/open cuff/sham load), with all rats receiving the same drug/cuff/gastric load treatment on the same day. Rats were tested at least twice under each experimental condition. The following six drug injection/gastric load conditions were tested with the cuff either open or closed for a total of 12 experimental conditions: saline/sham, ondansetron/sham, saline/5-ml load, ondansetron/5-ml load, saline/10-ml load, ondansetron/10-ml load.

**Experiment 3: 5-HT3 Receptor Blockade on Inhibition of Sham Feeding by CCK Combined with Gastric Distension**

A separate group of rats (n = 6) equipped with chronic gastric cannulas were trained to sham feed a 15% (wt/vol) sucrose solution. After overnight food deprivation, rats were removed from their cages at 0900, 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. Intraperitoneal drug administration consisted of saline, ondansetron (1.0 mg/kg), CCK (2.0 μg/kg), or a combined injection of CCK (2.0 μg/kg) and ondansetron (1.0 mg/kg). Five minutes after injection and immediately preceding presentation of sucrose, an 8-Fr Foley catheter (Bardex; Bard, Covington, GA) with an inflatable tip was fed through a drainage tube attached to the gastric fistula and advanced 0.5–0.7 cm into the lumen of the stomach. The catheter was held in place by a rubber band attached to the external end, which prevented movement from its original insertion position. The catheter was inflated with either 5 or 10 ml warmed 0.9% NaCl for a period of 10 min (13). Immediately after catheter inflation, rats were presented with a calibrated glass burette filled with 15% sucrose solution (wt/vol) and intake was recorded to the nearest 0.1 ml every 5 min for the ensuing 30 min. After the catheter was deflated (10 min into sham feeding) and removed from the stomach, rats were allowed to sham

**AJP-Regul Integr Comp Physiol • VOL 291 • JULY 2006 • www.ajpregu.org**
feed sucrose for the remaining 20 min. Each drug/distension treatment was given for at least two experimental days, separated by a minimum of 48 h, and was bracketed by a NaCl/no-distension condition. In all sham-feeding tests, gastric drainage was collected in plastic graduated cylinders placed beneath the cages, and the volume was recorded at experiment termination. In the event that the volume of fluid ingested was greater than the volume of gastric drainage or if gastric drainage did not occur within 15 s of the start of sham feeding, the data from that subject were discarded on the basis that the gastric fistula was not functioning properly (60).

**Experiment 4: 5-HT3 Receptor Blockade on Inhibition of Real Feeding by CCK Combined with An Intragastric Preload**

A separate group of overnight-fasted rats (n = 10) received an intraperitoneal injection containing either 0.9% NaCl, ondansetron (1.0 mg/kg), CCK (1.0 µg/kg), or a single combined injection of ondansetron and CCK. Five minutes after drug administration, rats received either a 5-ml load of 0.9% NaCl (wt/vol) instilled into the stomach via an orally inserted 8-Fr polyethylene intragastric tube, or the inserted tube without gastric load (sham load). A 5-ml volume was used to induce stomach distension before ingested sucrose. Immediately after gastric/sham load, rats were returned to their home cage and were presented with a calibrated glass burette filled with 15% sucrose (wt/vol) solution. Intakes were recorded to the nearest 0.1 ml every 5 min for 30 min. Each drug/gastric load treatment was separated by a minimum of 48 h and bracketed by a control condition (saline injection/sham load). Rats were tested at least twice under each condition, with all rats receiving the same drug/gastric load treatment on the same day. These methods are similar in design to those reported by Moran and McHugh (37) in monkeys, with the exception that we

**RESULTS**

**Experiment 1: 5-HT3 Receptor Blockade on Inhibition of Sham Feeding by CCK Combined with Intestinal Sucrose Infusion**

Two-way ANOVA revealed a significant main effect on sham sucrose intake at 30 min for drug treatment [F(3,165) = 20.50, P < 0.0001] and intestinal infusion [F(1,165) = 66.47, P < 0.0001]. There was no significant main effect of interaction between drug and intestinal infusion [F(3,165) = 0.57, P = 0.6346]. As Fig. 1 shows, sham intake in response to administration of CCK combined with intestinal infusion of 0.9% NaCl was significantly reduced at 30 min compared with control (26.4 ± 2.3 vs. 38.3 ± 1.0 ml; P < 0.0001). Ondansetron treatment alone did not produce any significant effect on sham intake compared with control (P > 0.05). Blockade of 5-HT3 receptors with ondansetron had no effect on CCK-induced suppression of sham intake (28.7 ± 2.3; P > 0.05). Intraintestinal 15% sucrose infusion reduced 30-min sham intake significantly compared with control (25.6 ± 3.2 vs. 38.3 ± 1.0 ml; P < 0.0001). However, ondansetron administration had no effect on intraintestinal sucrose-induced suppression of sham intake (27.8 ± 2.3; P > 0.05). When combined, CCK and intraintestinal sucrose significantly enhanced suppression of sham intake in an additive manner compared with control (18.3 ± 1.5 ml; P < 0.0001), intraintestinal sucrose alone (P = 0.0047), and CCK alone (P = 0.0005). Blockade of 5-HT3 receptors failed to attenuate the enhanced suppression of sham intake by CCK combined with intraduodenal sucrose infusion (18.7 ± 2.3; P > 0.05).

**Experiment 2: 5-HT3 Receptor Mediation of Gastric Distension-Induced Suppression of Real Feeding After Occlusion of the Pylorus**

Data from four rats were discarded from statistical analyses because of uncontrollable weight loss (resulting in euthanasia), development of pyloric strictures, and slipping of the cuff

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**Fig. 1. Combined administration of cholecystokinin (CCK; 2.0 µg/kg ip) with an intraduodenal 0.9% NaCl infusion significantly reduced sucrose intake compared with control [NaCl infusion/saline (SAL) vehicle injection] in sham-feeding rats. Likewise, intraduodenal infusion of 15% sucrose significantly reduced sham intake compared with control. When combined, CCK and intraduodenal sucrose significantly enhanced suppression of sham intake compared with control or either treatment alone. Blockade of 5-HT3 receptors by ondansetron (OND; 1.0 mg/kg ip) alone did not alter 30-min sham intake compared with control. When coadministered, OND failed to attenuate suppression of sham intake by CCK, intraduodenal sucrose infusion, or the enhanced suppression in sham intake by CCK combined with sucrose infusion. Bars with different letters are significantly different from each other (P < 0.05).**

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**Data and Statistical Analyses**

Data for each respective study were analyzed separately and expressed as means ± SE. For experiments 1, 3, and 4, sucrose intakes for all time points were analyzed by two-way repeated-measures ANOVA (rmANOVA), with drug injection and gastric/intestinal treatments as the main variables. For experiment 2, sucrose intakes were analyzed by three-way rmANOVA, with drug injection (NaCl, CCK, ondansetron), gastric load volume (0, 5, 10 ml) and cuff condition (open, closed) as the main variables. For all experiments, significant differences among treatment means (adjusted) were analyzed by pairwise Student’s t-test for planned comparisons, with P < 0.05 considered statistically significant. All analyses were made using PC-SAS (version 8.02; SAS Institute, Cary, NC) mixed procedure.
below the pylorus. For the remaining six rats, one-way ANOVA revealed an overall significant main effect for blood glucose over time after an oral glucose load $[F(3, 72) = 14.73, P < 0.001]$. Mean blood glucose for these six rats at 30 min (79.0 ± 4.2 mg/dl) and 60 min (69.8 ± 2.5 mg/dl) after occlusion of the pylorus and administration of an oral glucose load were not significantly different from each other ($P > 0.05$) or from blood glucose recorded just before receiving the oral glucose load (70.7 ± 3.0 mg/dl; $P > 0.05$). However, 30 min after the release of the pyloric cuff (90 min after oral glucose load), there was a significant rise in blood glucose (98.3 ± 3.8 mg/dl) compared with blood glucose values when the cuff was closed ($P \leq 0.004$). For these rats, the cuff was assessed to be effective at preventing gastric emptying. Therefore, the experimental data collected from six of the original ten animals were statistically analyzed.

Three-way ANOVA revealed a significant main effect on 30-min sucrose intake for gastric load $[F(2,54) = 5.25, P = 0.0083]$ and cuff condition $[F(1,54) = 88.28, P < 0.0001]$. There was no significant main effect of drug treatment $[F(1,54) = 2.24, P > 0.1406]$. Likewise, there was no significant interaction between drug and gastric load $[F(5,54) = 0.95, P > 0.46]$ or drug and cuff condition $[F(1,54) = 1.1, P = 0.2993]$. However, there was an overall significant interaction between gastric load and cuff condition $[F(2,54) = 7.88, P = 0.001]$, as well as drug, cuff condition, and gastric load $[F(7,54) = 2.93, P = 0.0112]$. As illustrated in Fig. 2, 30-min sucrose intake after saline injection was not significantly different, whether the cuff was in a closed (13.4 ± 0.6 ml) or open (16.1 ± 0.9 ml; $P > 0.05$) condition. Similarly, ondansetron alone did not produce any effect on sucrose intake whether the cuff was closed or open ($P > 0.05$). Administration of a 5-ml gastric NaCl load with the cuff closed significantly suppressed sucrose intake (9.1 ± 1.1 ml) compared with control (saline/cuff open/sham load; 16.1 ± 0.9 ml; $P < 0.001$) and intakes with the cuff closed in the absence of gastric load (saline/cuff closed/sham load; 13.4 ± 0.6 ml; $P = 0.006$). Instillation of a 5-ml NaCl load with the cuff open produced no significant effect on intake (15.8 ± 1.9 ml) compared with control (saline/cuff open/sham load; $P > 0.05$). When a 10-ml NaCl load was instilled with the cuff closed, sucrose intake was further suppressed (4.2 ± 0.4 ml) compared with control (saline/cuff open/sham load; $P < 0.0001$), as well as intake after a 10-ml NaCl load with the cuff open (15.3 ± 1.9 ml; $P < 0.0001$). Ondansetron administration significantly attenuated suppression of sucrose intake produced by a 10-ml NaCl load (6.8 ± 0.7 ml; $P = 0.037$), but not by a 5-ml NaCl load (9.0 ± 0.7 ml; $P > 0.05$) when the cuff was closed.

**Experiment 3: 5-HT3 Receptor Blockade on Inhibition of Sham Feeding by CCK Combined with Gastric Distension**

Two-way ANOVA revealed a significant main effect on sham sucrose intake at 30 min for drug treatment $[F(3,54) = 38.52, P < 0.0001]$, gastric distension $[F(2,54) = 35.98, P < 0.0001]$, and interaction between drug and gastric distension $[F(6,54) = 3.45, P = 0.0059]$. As Fig. 3 shows, sham intake in response to administration of CCK alone was reduced at 30 min compared with control (23.5 ± 3.1 vs. 40.8 ± 0.8 ml; $P < 0.0001$). Ondansetron treatment alone did not produce any significant effect on sham intake compared with control (40.0 ± 2.1; $P > 0.05$). Blockade of 5-HT3 receptors with ondansetron had no effect on CCK-induced suppression of sham intake (25.8 ± 2.9 vs. 23.5 ± 3.1 ml; $P > 0.05$). Gastric distension alone significantly reduced 30-min sham intake compared with control, 5 ml, and 10 ml volumes (40.8 ± 0.8, 28.2 ± 2.2, and 20.2 ± 2.2 ml, respectively; $P < 0.0001$). Ondansetron administration significantly attenuated 10-ml gastric distension-induced suppression of sham intake (30.5 ± 2.2 ml; $P = 0.001$). However, ondansetron was unable to produce a significant attenuation of intake after 5-ml gastric distension (33.6 ± 2.3 vs. 28.2 ± 2.2 ml for ondansetron + 5 ml and 5 ml alone, respectively; $P > 0.05$) When combined, CCK and 5-ml gastric distension enhanced suppression of intake compared with control, saline injection/sham distension condition (19.1 ± 1.7 vs. 40.8 ± 0.8 ml; $P < 0.0001$), and 5-ml gastric distension alone (19.1 ± 1.7 vs. 28.2 ± 2.2 ml; $P = 0.003$). However, when CCK was combined with 10-ml gastric distension, food intake was significantly suppressed with the pylorus occluded in response to a 0.9% NaCl gavage load (5 and 10 ml) in a volume-dependent fashion. Blockade of serotonin type-3 (5-HT3) receptors by OND (1.0 mg/kg ip) had no effect on suppression of intake by occlusion of the pylorus with either a sham or 5-ml NaCl gavage load. However, OND significantly attenuated the enhanced suppression of intake by a 10-ml gastric load after pyloric occlusion. Bars with different letters are significantly different from each other ($P < 0.05$).

![Fig. 2. Occlusion of the pylorus did not significantly suppress 30-min intake of 15% sucrose solution. Food intake was significantly suppressed with the pylorus occluded in response to a 0.9% NaCl gavage load (5 and 10 ml) in a volume-dependent fashion. Blockade of serotonin type-3 (5-HT3) receptors by OND (1.0 mg/kg ip) had no effect on suppression of intake by occlusion of the pylorus with either a sham or 5-ml NaCl gavage load. However, OND significantly attenuated the enhanced suppression of intake by a 10-ml gastric load after pyloric occlusion. Bars with different letters are significantly different from each other ($P < 0.05$).](http://ajpregu.physiology.org/)

**AJP-Regul Integr Comp Physiol • VOL 291 • JULY 2006 • www.ajpregu.org**
tension, this produced a further suppression in sham intake (12.0 ± 1.7 ml) compared with control (saline injection/sham gastric distension). When combined, CCK enhanced both 5- and 10-ml gastric distension-induced suppression of sham sucrose intake. Blockade of 5-HT3 receptors by OND (1.0 mg/kg ip) alone did not alter 30-min sham intake compared with control. OND, when coadministered with CCK was unable to attenuate CCK-induced suppression of 30-min sham intake. However, OND significantly attenuated suppression of sham intake by 10-ml gastric distension, as well as the enhanced suppression produced by CCK when combined with 10-ml distension. Bars with different letters are significantly different from each other (P < 0.05).

**Experiment 4: 5-HT3 Receptor Blockade on Suppression of Real Feeding by CCK Combined with Intragastric Load**

Two-way ANOVA revealed a significant main effect on sucrose intake at 30 min for drug treatment [F(3,62) = 48.65, P < 0.0001] and gastric load [F(1,62) = 25.65, P < 0.0001]. There was no significant main effect of interaction between drug treatment and gastric load [F(3,62) = 0.48, P > 0.05]. As illustrated in Fig. 4, systemic administration of CCK significantly reduced 30-min sucrose intake (6.3 ± 0.5 ml) compared with control (12.6 ± 0.3 ml; P < 0.0001). Ondansetron administration significantly attenuated CCK-induced suppression of real feeding (9.0 ± 0.9 vs. 6.3 ± 0.5 ml, for CCK and CCK/ondansetron, respectively; P = 0.034). Independently, 5-ml 0.9% NaCl gastric load produced a significant reduction in 30-min sucrose intake (10.9 ± 0.6 ml) compared with sham load (12.6 ± 0.3 ml; P < 0.05). Ondansetron administration alone did not produce any significant effect on sucrose intake compared with control (12.1 ± 0.5 vs. 12.6 ± 0.3 ml; P > 0.05). When CCK was combined with a 5-ml 0.9% NaCl gastric load, this resulted in an enhanced reduction of sucrose intake (4.6 ± 0.5 ml) compared with control (12.6 ± 0.3 ml; P < 0.0001) or NaCl gastric load alone (10.9 ± 0.6 ml; P < 0.0001). Blockade of 5-HT3 receptors significantly attenuated the enhanced suppression of food intake by CCK and gastric load (6.9 ± 0.6 vs. 4.6 ± 0.5 ml)

**Fig. 3.** Administration of CCK (2.0 µg/kg ip) significantly reduced sham 15% sucrose intake compared with control. Gastric distension significantly reduced sham intake at both 5- and 10-ml volumes of distension compared with control (sham distension). When combined, CCK enhanced both 5- and 10-ml gastric distension-induced suppression of sham sucrose intake. Blockade of 5-HT3 receptors by OND (1.0 mg/kg ip) alone did not alter 30-min sham intake compared with control. OND, when coadministered with CCK was unable to attenuate CCK-induced suppression of 30-min sham intake. However, OND significantly attenuated suppression of sham intake by 10-ml gastric distension, as well as the enhanced suppression produced by CCK when combined with 10-ml distension. Bars with different letters are significantly different from each other (P < 0.05).

**Fig. 4.** Intraperitoneal administration of CCK (1.0 µg/kg) alone significantly reduced intake of 15% sucrose compared with control (saline injection/sham gastric load). OND (1.0 mg/kg ip) alone did not alter 30-min intake compared with control. Administration of a 5-ml 0.9% NaCl load via oral gavage significantly suppressed intake compared with control (saline injection/sham gastric load). CCK administration before a 5-ml NaCl load produced an enhanced suppression in sucrose intake compared with control (saline injection/sham gastric load), CCK alone, or 5-ml NaCl gavaged load alone. OND significantly attenuated CCK-induced suppression of intake, as well as the enhanced suppression of intake produced by CCK combined with a gastric NaCl load. Bars with different letters are significantly different from each other (P < 0.05).
DISCUSSION

The results of these studies revealed that 5-HT3 receptor mediation of CCK-induced satiation requires gastric (i.e., filling and subsequent distension), but not intestinal feedback mechanisms. Specifically, blockade of 5-HT3 receptors had no effect on suppression of sham feeding by intraduodenal nutrients, CCK administration, or the combination of the two treatments. However, blockade of 5-HT3 receptors attenuated both suppression of sham feeding by gastric distension, as well as the enhanced suppression produced by the combination of gastric distension with CCK administration. Given our previous findings indicating that 5-HT3 receptors mediate CCK-induced satiation by delaying gastric emptying (21), current results demonstrate that CCK-induced activation of 5-HT3 receptors requires distension of the stomach. Finally, using both a sham feeding, as well as a real-feeding pyloric cuff preparation, our results demonstrate that 5-HT3 receptors mediate suppression of food intake by gastric distension in the absence of intestinal and cholecystokininergic negative feedback.

Activation of 5-HT3 receptors has been documented in numerous physiological and behavioral processes including control of gastric emptying (44), stimulation of pancreatic secretion (29), and intestinal transit (44, 62), as well as emetic (23) and cardiorespiratory reflexes (5, 56). This is not surprising considering the wide anatomical distribution of 5-HT3 receptors and the fact that 5-HT is released from various cell types, including enterochromaffin cells, platelets, endothelial cells, mast cells, and serotonergic neurons (3, 16). Of particular relevance is the role of 5-HT3 receptors in physiological response controlling for food intake. For example, we have previously shown that 5-HT3 receptors mediate suppression of food intake by intestinal nutrients (48) and CCK (21, 22), as well as other physiological functions controlled by CCK, such as gastric emptying (21). Thus 5-HT3 receptors are not only critical in mediating gastric distension-induced suppression of food intake but also in the communication between the intestine and the stomach by intensifying the strength of the satiety signal. The mechanism by which this occurs is not entirely clear. However, gastric distension has been shown to cause 5-HT release (33). Therefore, these findings provide evidence that one mechanism by which ondansetron attenuates suppression of sucrose intake by gastric distension is via the release of 5-HT in response to gastric distension and subsequent blockade of 5-HT3 receptors. By combining the sham-feeding preparation with intestinal infusion of the same solution as the ingestate (15% sucrose), we were able to eliminate only gastric feedback and show that intestinal mechanisms do not contribute to 5-HT3 receptor’s role in satiation by CCK. Therefore, we examined the independent participation of 5-HT3 receptors in mediating suppression of intake by gastric distension when the ingestate was confined to the stomach by using an inflatable cuff. We showed that blockade of 5-HT3 receptors attenuates suppression of real feeding by stomach distension in the absence of intestinal feedback. These findings, however, do not negate participation of 5-HT3 receptors in mediating intraintestinal nutrient-induced suppression of food intake. Indeed, we and others have shown that 5-HT3 receptors mediate suppression of food intake by both intestinal lipid (4, 49) and carbohydrate solutions (48). In view of the current data, gastric feedback is also necessary for 5-HT3 receptor mediation of suppression of real feeding by nutrients. However, direct investigation of this notion remains to be tested.

The final experiments focused on participation of 5-HT3 receptors in suppression of food intake by CCK when combined with gastric distension. We were able to demonstrate that blockade of 5-HT3 receptors attenuates the enhanced suppression of sham intake by CCK when combined with gastric balloon distension and removal of intestinal feedback by the ingestate. Finally, we showed that 5-HT3 receptors mediate the enhanced satiation produced by CCK when combined with a gastric load in a real feeding paradigm. These findings support our previous reports that 5-HT3 receptor mediation of CCK-induced satiation requires inhibition of gastric emptying (21) and show that gastric distension is required for 5-HT3 receptor mediation of CCK-induced satiation.

We observed that the most pronounced effect of ondansetron on suppression of intake by gastric distension occurred when large gastric loads (e.g., 10 ml) were used. Therefore, it appears that 5-HT3 receptors are involved in satiation primarily when stomach distension is greater, such as toward meal termination. The fact that 5-HT3 receptors participate in gastric distension-induced satiation to a greater extent when a larger volume of balloon distension was applied is supported by our sham-feeding data. Consequently, recruitment of 5-HT3 receptors in mediating CCK-induced satiation most likely occurs with a greater degree of gastric distension in response to inhibition of gastric emptying by CCK. This is supported by studies of Muurahainen et al. (38) who demonstrated that intakes of a test meal in humans were significantly lower when CCK was given after a 500-g but not a 100-g soup preload. This is not to say, however, that 5-HT3 receptors are not activated by smaller volumes of stomach distension. In fact, using an ex vivo stomach preparation, Mazda et al. (33) demonstrated that 5-HT3 receptor activation occurs with as little as 3 ml of gastric distension. Indeed, our current results show that suppression of intake by the combination of a 5-ml NaCl load with CCK administration was significantly attenuated by ondansetron. Furthermore, although not significant, our sham-feeding findings showed that blockade of 5-HT3 receptors attenuates 5-ml distension-induced suppression of sham intake. One plausible explanation for this phenomenon could be an increase in endogenous 5-HT secretion in response to an amplified stomach distension, since it is well known that enterochromaffin cells can function as gastric mechanoreceptors and release 5-HT in response to mechanical pressure (15, 61). 5-HT3 receptors are also known to mediate nausea or acute emesis (23, 28). Therefore, it could be suggested that a 10-ml distension volume could have produced nausea symptoms. However, the 5- and 10-ml volumes of distension applied in these studies are within the range of physiological distension, and well below postprandial gastric capacity and therefore are not likely noxious. Moreover, though not quantified, we did not observe any malaise-like behavior after administration of ondansetron or in response to gastric distension at any volume tested. In fact, in both real- and sham-feeding studies, rats continued to consume sucrose in response to all volumes of distension. Nonetheless, we cannot entirely rule out...
the possibility that there may, in fact, be some subthreshold noxious response to gastric distension activating 5-HT3 receptors.

In addition to demonstrating the involvement of 5-HT3 receptors in suppression of intake by gastric distension, the results from the pyloric cuff experiment confirmed previous findings showing that gastric fill contributes significantly to the stomach’s role in satiation for food (25). In our study, however, sucrose intake was slightly reduced when the cuff was inflated and ingested fluid was confined to the stomach. Davis et al. (12) showed that occlusion of the pylorus decreases 30-min intake in rats who consume large volumes of the test meal (>15 ml) when their pylorus is open. Occlusion of the pylorus does not reduce intake in rats consuming smaller volumes of ingestate (42, 52). Given the fact that average baseline sucrose intake in rats who consume large volumes of the test meal and ingested fluid was confined to the stomach, Davis et al. sucrose intake was slightly reduced when the cuff was inflated. These findings showing that gastric fill contributes significantly to the results from the pyloric cuff experiment confirmed previous receptors in suppression of intake by gastric distension, the noxious response to gastric distension activating 5-HT3 receptors. In addition, these findings demonstrate that blockade of 5-HT3 receptors attenuates gastric distension-induced suppression of both real and sham feeding and that this effect occurs in response to a larger volume of stomach distension. In a broader context, since CCK-1 receptor activation occurs in response to nutrients entering the small intestine and 5-HT3 receptor activation occurs via gastric distension, it appears that CCK-1 and 5-HT3 receptors participate in inhibition of food intake by integrating both intestinal and gastric satiating signals.

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