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Serotonin-type 3 receptors mediate intestinal Polycose- and glucose-induced suppression of intake

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Savastano, David M., Melissa Carelle, and Mihai Covasa. Serotonin-type 3 receptors mediate intestinal Polycose- and glucose-induced suppression of intake. Am J Physiol Regul Integr Comp Physiol 288: R1499–R1508, 2005. First published February 17, 2005; doi:10.1152/ajpregu.00745.2004.—Ondansetron, a selective serotonin-type 3 (5-HT3) receptor antagonist, was used to test the hypothesis that duodenal infusion of isosmotic solutions of Polycose or its hydrolytic product glucose suppressed intake through 5-HT3 receptors. Polycose suppressed sucrose intake across both concentrations infused (132 mM, 7.6 ± 0.6 ml; 263 mM, 2.3 ± 0.5 ml), compared with intake under control conditions (12.6 ± 0.3 ml, P <0.001). Pretreatment with 1.0 mg/kg ondansetron attenuated reduction of sucrose intake only by the highest concentration of Polycose (4.6 ± 0.8 ml, P = 0.004). Dose-response testing revealed that suppression of food intake by 263 mM Polycose was equally attenuated by ondansetron administered at 1.0, 2.0, and 5.0 mg/kg but not when given at 0.125, 0.25, and 0.5 mg/kg. Acarbose, an α-glucosidase inhibitor, attenuated Polycose-induced suppression of food intake, and pretreatment with 1.0 mg/kg ondansetron had no further effect. Suppression of intake after 990 mM glucose but not mannitol infusion was attenuated by pretreatment with 1.0 mg/kg ondansetron. The competitive SGLT1 inhibitor, phloridzin, had no effect on 60-min 990 mM glucose-induced suppression of intake or the ability of ondansetron to attenuate this suppression of intake. Conversely, glucose-induced suppression of intake was attenuated by phloridzin at earlier time points and further attenuated when rats were pretreated with 1.0 mg/kg ondansetron. Ondansetron administration alone had no effect on intake at any dose tested. We conclude that 5-HT3 receptors participate in the inhibition of food intake by intraduodenal infusion of carbohydrate solutions through a postabsorptive, preabsorptive mechanism. nutrient absorption; food intake; satiation

THE PRESENCE OF NUTRIENTS in the intestine elicits signals essential to feedback control of ingestion. Considerable advances have been made in understanding the mechanisms by which nutrients in the intestine suppress intake in a number of species (30, 33), including rats (42, 51, 53) and humans (74). For example, it is well documented that intestinal carbohydrates reduce food intake in a dose-responsive manner (53, 76) by preabsorptive (63, 78) as well as postabsorptive (71) factors, largely through vagal afferent pathways (78, 79). However, the complete mechanism(s) by which intestinal oligosaccharides or monosaccharides is perceived to elicit negative feedback control of intake is not known. Administration of the α-glucosidase inhibitor, acarbose, attenuates reduction of food intake and c-Fos-immunoreactivity by maltotriose (62, 63). Moreover, inhibition of sodium-dependent glucose transporter 1 (SGLT1) by intestinal phloridzin infusion fails to attenuate suppression of food intake and vagal afferent activation by maltotriose or glucose, even though the transport of glucose to the blood is almost entirely blocked (62, 63). These findings suggest that reduction of food intake by intestinally infused carbohydrates requires hydrolysis to glucose but that transport of glucose to the blood is not necessary. Because vagal afferent terminals do not penetrate between epithelial cells or protrude into the lumen (3), the receptive mechanism by which intestinal glucose elicits a reduction of intake seems to be localized on the luminal side of the intestinal mucosa, outside of the direct neural afferent activation locale. Therefore, a paracrine or endocrine product of intestinal origin must interfere in vagal afferent signaling. The identity of such a product or products is not certain; however, neuroactive modulators released in response to intestinal stimulation are the most obvious candidates (60). Duodenal carbohydrate solutions stimulate the release of several putative gastrointestinal (GI) hormones and neuropeptides that contribute to the negative feedback control of food intake (62), one of which is the brain-gut indoleamine serotonin (5-hydroxytryptamine, 5-HT) (21, 46, 49, 52, 55, 80).

The serotonergic system has been well studied for its important role in the control of food intake. For example, administration of agonists activating postsynaptic 5-HT3 receptors produces marked reductions in food intake (4, 64, 68). Likewise, administration of 5-HT receptor antagonists has been shown to elicit food intake in rats (20, 26). Systemic serotonergic activity has been shown to induce an anorectic response through activation of a number of receptors, including the excitatory, ligand-gated, cation channel 5-HT3 receptor (1, 37, 80). 5-HT3 receptors are predominantly located on terminals of vagal afferent fibers (10, 28, 34, 61) in addition to distinct subpopulations in the brain (9, 56, 58). This receptor class has been shown to be involved in mediating GI functions such as gastric emptying, pancreatic secretion, and colonic transit (29, 61, 80) and have also been shown to be involved in the control of intake under a variety of feeding paradigms. For instance, 5-HT3 receptors mediate anorectic responses to dietary amino

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acid deficiency (1, 2, 19), as well as peripheral CCK adminis-
tration (17, 38, 39). There is also some preliminary data
demonstrating that 5-HT3 receptors mediate suppression of
intake following intestinal preloads of a nutritionally complete
meal (7). Although these findings suggest that endogenous
5-HT, in response to intraintestinal nutrients, participates in the
control of food intake via 5-HT3 receptor activation, it is not
clear whether specific nutrient classes or their digestive by-
products activate the 5-HT3 receptor to bring forth short-term
reductions in food intake.

Conversely, the products of carbohydrate digestion, in particular,
have been shown to mediate feeding-related GI func-
tions via 5-HT3 receptor activation. Functional evidence indi-
cates that 5-HT3 receptors are involved in initiation of intesti-
nal feedback inhibition of gastric emptying (61) and stimula-
tion of pancreatic secretion (46) in response to intestinal perfusion
with glucose and maltose solutions, respectively. Furthermore,
electrophysiological studies have shown that activation of vagal afferent fibers by the products of carbohydrate digestion
is abolished by the administration of a 5-HT3 receptor antag-
onist (80). Given this evidence, it is conceivable that 5-HT3
receptors are functionally important in mediating intestinal
feedback inhibition of food intake in response to carbо-
drates. However, there are no reports that directly demonstrate
whether a highly selective 5-HT3 receptor antagonist can attenuate the reduction of intake resulting from complex carbo-
drates (glucose polymers) or monosaccharides (glucose)
delivered into the proximal intestine.

Therefore, our present studies were designed to investigate
participation of 5-HT3 receptors in carbohydrate-induced sup-
pression of food intake. Specifically, we examined whether the
reduction of intake after an intraintestinal infusion of Polycose
(glucose polymers) or monosaccharides (glucose) could be attenuated via blockade of 5-HT3 receptors. Additionally, we tested the contribution of luminal Polycose hydrolysis and free glucose on 5-HT3-receptor-me-
diated suppression of intake. Finally, we explored whether glucose absorption was required to suppress food intake
through 5-HT3-receptor activation.

METHODS

Animals and Surgical Preparation

Adult (250–350 g) male Sprague-Dawley rats (Harlan, Indianapolis, IN) were housed individually in hanging wire bottom cages and adapted to a 12:12-h light-dark cycle (lights on at 0600) in a tempera-
ture-controlled vivarium. Rats had ad libitum access to pelleted
rodent chow (Purina, 5001) and water, except as indicated in the
experimental procedure when they were deprived of food but not
water overnight (17 h). Once acclimated to laboratory conditions, rats were fitted with chronic duodenal catheters, consisting of a 22-cm length of silicone rubber tubing (0.025 in. (ID), 0.047 in. (OD), Dow
Corning, Midland, MI) as described previously by Yox and Ritter
(78). Catheters were inserted 2-cm distal to the pylorus and advanced
6 cm within the duodenal lumen in an aboral direction. The exposed
catheter end of the catheter exited through a skin incision over the dorsal
aspect of the cranial portion of the neck and was occluded with a stainless steel wire (obturator), which was removed only for flushing of the catheter and infusions. A minimum of 7 days was allowed for recovery, during which rats attained their preoperative body weights before the experiments began. Animal protocols were approved by
The Pennsylvania State University Institutional Animal Care and Use
Committee.

Treatments

5-HT3 receptor blockade. The selective 5-HT3 receptor antagonist
ondansetron HCl (a gift from GlaxoSmithKline, Barnard Castle, UK)
was diluted in physiological saline to 0.125, 0.25, 0.5, 1.0, 2.0, and 5.0
mg/ml dilutions. In all experiments, ondansetron was administered
intraperitoneally (1.0 ml/kg), whereas control treatment consisted of
an intraperitoneal (ip) injection of physiological saline.

Intestinal infusions. Polycose (Ross Laboratories, Columbus, OH)
was diluted with distilled water to 132 and 263 mM concentrations.
Acarbose, an α-glucosidase inhibitor, was isolated via centrifugation
of dissolved Precose tablets (100 mg; Bayer; Burns Veterinary) and
made to a pH of 7.35–7.40.

General Procedures

Overnight food-deprived (17 h) rats were given an intraperitoneal injection of either saline or ondansetron 5 min before the initiation of the intraintestinal infusion. Infusates were delivered at a rate of 0.4
ml/min for 20 min. This infusion rate has been shown to be within the
physiological range of gastric emptying (42). After infusion ceased, rats were returned to their home cages and immediately presented
with 15% sucrose in a graduated drinking burette; intake was mea-
sured to the nearest 0.1 ml over the subsequent 60 min. Treatments
(ondansetron injection and/or nutrient infusion) were separated by
saline-saline control tests. In the studies presented here, the effect
of ondansetron on carbohydrate-induced suppression was significant only during the latter half of the 60-min period (Fig. 6).

Experiments

Four experiments were performed. Experiment 1 tested the hypo-
thesis that the reduction of feeding in response to intraintestinal Poly-
cose infusion occurs via a 5-HT3-receptor-mediated mechanism. One
group of rats (n = 6) received an injection of saline or ondansetron
(1.0 mg/kg) followed by intraintestinal infusion of either saline or 132
or 263 mM Polycose administered in ascending order of osmotic
concentration. In a separate group of rats (n = 10), the effects of
ondansetron across a range of doses (0.125, 0.25, 0.5, 1.0, 2.0, and 5.0
mg/kg) on 263 mM Polycose-induced suppression of sucrose intake
were examined.

Experiment 2 examined whether the products of luminal glucose
polymer hydrolysis were necessary to reduce food intake via 5-HT3
receptor activation. Rats previously used for infusion experiments
(n = 12) received an injection of saline or ondansetron (1.0 mg/kg)
followed by infusion of solutions that included the α-glucosidase
inhibitor acarbose (0.2%) either alone or in combination with 263 mM
Polycose.

To further assess 5-HT3 receptor participation in the reduction of
food intake in response to products of glucose polymer hydrolysis,
experiment 3 was performed to test the hypothesis that free luminal glucose suppresses intake through 5-HT3 receptors. Infusions up to this point were delivered in isosmotic concentrations given that high luminal osmolarity alone has been shown to release 5-HT (52) and elicit 5-HT3 receptor activation (46, 61). Because glucose has a molecular weight approximately five times less than that of Polycose, it is not possible to infuse glucose across increasing satiating concentrations while maintaining isotonicity. Therefore, to control for the osmotic properties and establish whether the satiating effect was specific to glucose, the same osmotic concentrations of mannitol were also tested. In experiment 3, experimentally naive rats (*n* = 6) were infused with glucose or mannitol in 0, 308, 694, or 990 mM concentrations after an injection of saline or ondansetron.

The final experiment, experiment 4, explored whether 5-HT3 receptors participate in the suppression of intake when active, Na+-dependent absorption of duodenal glucose is inhibited using phloridzin, the competitive SGLT1 inhibitor. Phloridzin is a useful tool in the study of intestinal glucose transport because it competitively binds SGLT1 and inhibits its activity (44). In this experiment, rats (*n* = 12) were injected with either saline or ondansetron (1.0 mg/kg) before duodenal infusion of either saline, 990 mM glucose, phloridzin (0.39%), or glucose combined with phloridzin.

To determine phloridzin’s efficacy in intestinal glucose transport inhibition, blood glucose levels were measured in response to infusions of saline, 990 mM glucose, phloridzin (0.39%), or glucose combined with phloridzin. Blood glucose was measured in a drop of tail blood obtained serially from each rat at 0, 20, 30, 45, 60, and 120 min relative to the start of the infusion, using a handheld glucometer (LifeScan, Milpitas, CA).

**Analyses of Results**

Data for each respective experiment were analyzed separately. Sucrose intake (ml) measurements for each rat were subjected to a two-way repeated-measures ANOVA (rmANOVA), with drug injection and intestinal infusate as independent variables, using PC-SAS (version 8.02, SAS Institute, Carey, NC) mixed procedure. Blood glucose data (mg/dl) for each rat were subjected to a two-way rmANOVA, with drug injection and time as independent variables. Significant differences among treatment means (adjusted) were analyzed by pairwise *t*-tests for planned comparisons, with *P* < 0.05 considered statistically significant.

**RESULTS**

In all experiments, when rats received ondansetron prior to intestinal saline infusion, intake was not significantly different from control (saline injection-saline infusion) at any time (see Figs. 1 and 3–5 and Table 1).

### Table 1. Effects of ondansetron on 263 mM Polycose-induced inhibition of food intake

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<td></td>
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<td>Saline/saline</td>
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<td>Saline/Polycose</td>
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<tr>
<td>Ondansetron/Polycose</td>
<td>4.8±0.8*</td>
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Values are means ± SE 60-min 15% sucrose intakes (ml) in overnight food-deprived rats (*n* = 10). Infusion with 263 mM Polycose produced a significant reduction of 15% sucrose intake. Ondansetron administered at 1.0, 2.0, and 5.0 mg/kg each significantly attenuated Polycose-induced suppression of intake. *Significantly different from saline-saline treatment within a column (*P* < 0.05). †Significantly different from saline-Polycose treatment within a column (*P* < 0.05).

### Effects of 5-HT3 Receptor Blockade on Polycose-Induced Inhibition of Food Intake

rmANOVA demonstrated a significant main effect of injection ([F(1,121) = 5.32, *P* = 0.02] and a significant main effect of infusion ([F(2,121) = 124.36, *P* < 0.0001]) but not a significant interaction between injection and infusion ([F(2,121) = 1.54, *P* = 0.22]. As illustrated in Fig. 1, infusion of 132 mM Polycose significantly suppressed 60-min intake (7.6 ± 0.6 ml) compared with intake under control conditions (12.6 ± 0.3 ml, *P* < 0.001).

Pretreatment with ondansetron (1.0 mg/kg) did not significantly alter this suppression (8.3 ± 0.4 ml, *P* = 0.45). Infusion with 263 mM Polycose significantly suppressed intake (2.3 ± 0.5 ml, *P* < 0.001) compared with saline. This suppression was significantly attenuated after pretreatment with ondansetron (4.6 ± 0.8 ml, *P* = 0.004).

Table 1 shows the effects of treatment with ondansetron (0.125–5.0 mg/kg) on 263 mM Polycose-induced inhibition of 60-min sucrose intake. Infusion with 263 mM Polycose produced a significant reduction of sucrose intake ([F(1,195) = 976.74, *P* < 0.001]. rmANOVA also demonstrated an overall effect of injection ([F(6,195) = 3.85, *P* = 0.0012] as well as a significant interaction between injection and infusion ([F(6,195) = 4.42, *P* < 0.001]. The degree to which suppression of intake was attenuated by each dose of ondansetron is illustrated in Fig. 2 via mean difference scores. The difference score for each rat was calculated as intake following treatment with ondansetron and Polycose minus intake following treatment with saline and Polycose. One-way rmANOVA revealed no significant differences between the degree to which ondansetron attenuated Polycose-induced suppression of food intake across all doses tested ([F(5,44) = 0.286, *P* = 0.91]). However, only 1.0, 2.0, and 5.0 mg/kg ondansetron significantly attenuated Polycose-induced suppression of intake (Table 1).

### Inhibition of Luminal Glucose Polymer Hydrolysis Accompanied by 5-HT3 Receptor Blockade

There was a significant main effect of infusion on intake ([F(3,147) = 105.52, *P* < 0.0001]. However, rmANOVA did not reveal a significant main effect of injection ([F(1,147) = 2.57, *P* = 0.11] or an overall interaction between injection and infusion ([F(3,147) = 2.10, *P* = 0.103]. Rats infused with 263 mM Polycose significantly suppressed 60-min sucrose intake (2.1 ± 0.6 ml) compared with intake under control conditions (12.8 ± 0.3 ml, *P* < 0.0001). This suppression was significantly attenuated after pretreatment with 1.0 mg/kg ondansetron (4.4 ± 0.9 ml, *P* = 0.019; Fig. 3). A combined infusion of...
0.2% acarbose and 263 mM Polycose resulted in a significant suppression of sucrose intake (10.4 ± 0.8 ml, P = 0.0015) compared with control. Pretreatment with ondansetron did not significantly alter this suppression (10.0 ± 0.9 ml, P = 0.65). Infusion of acarbose alone resulted in sucrose intake that was not significantly different from control when rats were injected with saline (12.2 ± 0.9 ml, P = 0.44) or ondansetron (13.3 ± 0.6 ml, P = 0.22).

**Effects of 5-HT₃ Receptor Blockade on Glucose-Induced Inhibition of Food Intake**

rmANOVA failed to demonstrate a significant main effect of injection [F(1,293) = 0.9, P = 0.34] but demonstrated a significant main effect of infusion [F(6,293) = 50.66, P < 0.0001] as well as an overall interaction between injection and infusion [F(6,293) = 4.63, P = 0.0002]. As illustrated in Fig. 4, infusion of 308 mM glucose resulted in 60-min sucrose intake (12.5 ± 0.5 ml) that was less than intake under control conditions (13.4 ± 0.2 ml), but this difference was not significant (P = 0.052). Pretreatment with 1.0 mg/kg ondansetron did not significantly change this intake (12.3 ± 0.5 ml, P = 0.84). Similarly, sucrose intake was not suppressed when rats were infused with 308 mM mannitol (14.7 ± 0.6 ml, P = 0.09). Pretreatment with ondansetron did not alter this intake (13.0 ± 0.8 ml, P = 0.15). Infusion of 694 mM glucose significantly suppressed intake (10.0 ± 0.7 ml, P < 0.0001) compared with control, and pretreatment with ondansetron did not significantly alter this suppression (10.4 ± 1.3 ml, P = 0.69). Likewise, infusion of 694 mM mannitol suppressed intake (11.3 ± 1.1 ml, P = 0.001) compared with control. This suppression was not changed by pretreatment with ondansetron (12.2 ± 0.7 ml, P = 0.43). The 990 mM glucose infusion markedly suppressed sucrose intake (0.4 ± 0.1 ml, P < 0.001), which was significantly attenuated by pretreatment with ondansetron (4.4 ± 0.7 ml, P = 0.005). Infusion with 990 mM mannitol also significantly suppressed sucrose intake (5.0 ± 0.8 ml, P < 0.001), but this suppression was not altered following pretreatment with ondansetron (4.8 ± 1.2 ml, P = 0.89).

**Effects of 5-HT₃ Receptor Blockade on Glucose-Induced Reduction of Food Intake While Inhibiting Active Intestinal Glucose Transport**

Significant main effects of injection [F(1,109) = 4.47, P = 0.037] and infusion [F(3,109) = 156.47, P < 0.0001] and significant interaction between injection and infusion [F(3,109) = 4.53, P = 0.005] on intake were demonstrated by rmANOVA. As illustrated in Fig. 5, infusion with the competitive SGLT₁ inhibitor phloridzin produced a 60-min intake (17.3 ± 1.4 ml) result similar to that of control (18.0 ± 0.7 ml, P = 0.3). This intake was not altered by pretreatment with 1.0 mg/kg ondansetron (15.7 ± 1.3 ml, P = 0.16). Infusion of 990 mM glucose significantly sup-
pressed sucrose intake (4.3 ± 1.0 ml, *P < 0.001) compared with intake following control treatment. The suppression of intake following 990 mM glucose infusion was significantly attenuated when rats were pretreated with ondansetron (7.7 ± 1.0 ml, *P = 0.002). When rats were infused with phloridzin in combination with 990 mM glucose, sucrose intake was significantly suppressed (4.9 ± 1.0 ml, *P < 0.001) compared with control but not different from glucose infusion alone (*P = 0.58). The suppression of intake resulting from glucose plus phloridzin infusion was significantly attenuated when rats were pretreated with ondansetron (7.5 ± 1.3 ml, *P = 0.018).

Figure 6 illustrates the 60-min feeding period in 5-min intervals for the most relevant treatments. Infusion of glucose significantly suppressed sucrose intake for the entire 60-min period (*P < 0.0001). Suppression of intake following glucose infusion was significantly attenuated beginning at 40 min when rats were pretreated with ondansetron (*P ≤ 0.01). Duodenal infusion of phloridzin in combination with glucose significantly attenuated glucose-induced suppression of intake starting at 5 min and lasting until 55 min (*P = 0.009), at which time glucose-infused rats began to increase intake. Treatment with ondansetron enhanced this attenuation of glucose-induced suppression of intake in the presence of phloridzin for the entire 60-min intake that was measured (*P ≤ 0.01).

Blood glucose data indicative of phloridzin function are presented in Table 2. Duodenal infusion (during the first 20 min) of 990 mM glucose significantly elevated blood glucose compared with saline infusion at all time points measured (*P < 0.05). Significant elevation of blood glucose, compared with saline infusion, was achieved at 45, 60, and 120 min when glucose was infused in combination with 0.39% phloridzin (*P < 0.05). Phloridzin attenuated glucose-induced elevation in blood glucose from 20 through 45 min (*P < 0.05).

**DISCUSSION**

This study provides evidence that 5-HT3 receptors participate in carbohydrate-induced reduction of intake. More specifically, blockade of 5-HT3 receptors with ondansetron attenuated the reduction of intake following intraintestinal Polycose infusion. Additionally, by inhibiting luminal glucose polymer hydrolysis, we demonstrated that the products of hydrolysis are indeed necessary for 5-HT3 receptors to mediate Polycose-induced suppression of intake. Finally, we showed that ondansetron attenuated suppression of food intake in response to intraintestinal infusion of free luminal glucose and that this response was not altered by blocking active glucose absorption.

In a broader context, the findings of this study corroborate those from other laboratories (1, 7, 8, 17) as well as our own (38, 39), demonstrating that 5-HT3 receptors are involved in the negative feedback control of intake. For example, systemic administration of ondansetron has been shown to reverse suppression of intake resulting from duodenal infusion of a complete meal (Ensure) (7). Likewise, the inhibitory effect on the intermeal interval following an intestinal fat infusion was partially mediated by 5-HT3 receptors (8). To our knowledge, this is the first study to demonstrate participation of 5-HT3 receptors in suppression of food intake by complex, as well as simple, carbohydrates.

Nutrients entering the intestine present a composite of coligative and chemical properties to the intestinal sensory system, which act in concert to generate satiation. Each macronutrient constituent might influence food intake by distinct mechanisms. It is known that much of the suppression of food intake after intestinal nutrient infusion is mediated by vagal sensory neurons (78, 79). However, it is uncertain whether vagal afferents can respond directly to nutrients in the extracellular space or whether responses to nutrients are mediated by substances released from the intestinal mucosa or enteric neurons (36). There is support for the hypothesis that endocrine cells in the gut act as an intermediate between intestinal stimuli and vagal afferent termini by releasing their contents in response to mechanical and chemical stimulations of the intestinal wall (59, 60). For example, 5-HT is found in abundance in enterochromaffin cells of the GI mucosal epithelia and the enteric neurons (32) and is released in response to luminal...
disaccharide solutions (46, 49, 55, 80) as well as glucose, but not mannitol, in a concentration-dependent fashion (21, 43, 52). Although some evidence shows that vagotomy does not change or actually enhances the anorectic effect of exogenous 5-HT (22, 27), other data suggest that peripheral 5-HT acts as a signal molecule in carbohydrate-induced inhibition of food intake, most likely through vagal afferent activation. For example, electrophysiological studies show that endogenously released 5-HT plays a major role in signal transmission evoked by luminal carbohydrates to stimulate vagal fibers (47, 80), including nodose neurons (48, 50, 80). Vagal sensory fibers, which are important in the negative feedback cascade to inhibit food intake (78, 79), are likely involved in 5-HT3-receptor-mediated suppression of intake observed in our study. 5-HT3 receptors are amply localized within the gut, specifically on vagal sensory fibers innervating the duodenum (31, 34, 58, 61), and have been shown to be involved in mediating carbohydrate-induced inhibition of gastric emptying (61). In addition, electrophysiological evidence reveals that stimulation of vagal sensory neurons with intestinal maltose is inhibited by administration of a 5-HT3 antagonist (80). Taken as a whole, these findings suggest that intestinal carbohydrates elicit 5-HT release and subsequent activation of 5-HT3 receptors present on vagal sensory pathways as part of a feedback cascade to inhibit food intake.

In the first experiment, we did not observe a robust dose response effect of ondansetron on Polycose-induced suppression of intake. Our results showed that 1.0 mg/kg was the lowest dose of ondansetron able to significantly attenuate 263 mM Polycose-induced suppression of intake and that higher doses (2.0 and 5.0 mg/kg) did not enhance the effect. The lower doses of ondansetron tested (0.125–0.5 mg/kg) all slightly attenuated Polycose-induced suppression of intake, but statistical significance was not reached. Thus, in the presence of intestinal Polycose, ondansetron (0.125–5.0 mg/kg) exhibited a relatively flat dose-response function, with the 1.0 mg/kg dose having the maximal threshold effect on blocking 5-HT3 receptors and doses five times higher producing no further effects. Studies examining participation of 5-HT3 receptors in nutrient-induced satiation are scant. In addition, the few experiments that have investigated the role of 5-HT3 receptors in negative feedback inhibition of intake or feeding-related physiological functions using ondansetron have been single-dose studies. To our knowledge, this is the first study demonstrating the involvement of 5-HT3 receptors in carbohydrate-induced suppression of food intake. Previous studies that have examined the effects of ondansetron alone on several functions, including feeding behavior, have yielded conflicting results. For example, a novel, inverted-U dose-response function has been reported in studies that have attempted to characterize the actions of ondansetron on anxiolytic activity or benzodiazepine withdrawal (35, 41). In one of these studies in which food consumption was a secondary outcome measured, 0.01–1.0 mg/kg doses of ondansetron treatment alone produced no effect on food intake (35). Furthermore, studies examining the effects of much lower doses of ondansetron on food intake are equally inconsistent. For instance, in one study, administration of ondansetron (30 and 100 μg/kg) significantly increased sweetened mash intake and decreased 3% sucrose solution intake (10 and 30 μg/kg) in non-food-deprived rats (14); in a subsequent study, similar doses of ondansetron (3.0 to 30 μg/kg) had the opposite effect [i.e., significantly reduced sweetened mash intake (72)]. The reason for such discrepancies is not immediately clear. It is apparent from our results, however, that blockade of 5-HT3 receptors with at least 1.0 mg/kg ondansetron maximally attenuated the carbohydrate-induced suppression of intake.

The finding that blockade of 5-HT3 receptors only attenuated the carbohydrate-induced suppression of intake to a relatively small degree supports the likelihood that either other postsynaptic 5-HT receptors are involved in the mediation of this signal and/or that redundant signaling mechanisms are involved. In fact, Raybould et al. (61) found that both glucose-induced and mannitol-induced inhibitions of gastric emptying are abolished by pretreatment with tropisetron, a receptor antagonist that exhibits affinity for both 5-HT3 and 5-HT4 receptors (70). These results suggest the possibility that both receptor types cooperate to mediate intestinal feedback inhibi-

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<td>120</td>
<td>62.7±2.4</td>
<td>53.3±0.6</td>
<td>76.4±7.6*</td>
<td>85.0±1.8†</td>
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Values are means ± SE blood glucose in mg/dl. Duodenal infusion (during the course of 0–20 min) of 990 mM glucose significantly elevated blood glucose compared with saline infusion at all time points. Compared with saline infusion, significant elevation of blood glucose occurred at 45, 60, and 120 min when glucose, in combination with the competitive SGLT1 inhibitor phloridzin (0.39%), was infused. Phloridzin attenuated the glucose-induced elevation in blood glucose from 20 through 45 min. *Significantly different from saline infusion for a given time point (P < 0.05). †Significantly different from glucose infusion for a given time point (P < 0.05).
tion mechanisms in response to carbohydrates. Several studies have also shown that inhibition of intake by carbohydrate is mediated by other signals, one of which is CCK. Specifically, selective antagonists for the CCK type-1 (CCK-1) receptors attenuate the feeding inhibition (5, 57, 77) and dorsal hindbrain expression of c-Fos (73), resulting from duodenal carbohydrate solutions. Additionally, rats lacking CCK-1 receptors are insensitive to the suppressive effects of duodenal glucose infusions (16). Although both serotonergic and cholecystokininergic systems work independently to reduce food intake, their actions impinge on each other at several levels to modify intake in an integrated control of food intake. Original investigations (11–13, 66) have suggested that CCK's inhibitory action on food intake is potentiated by 5-HT and vice versa. More recently, Li et al. (48) reported that some but not all CCK-responsive vagal neurons are also responsive to intraluminal 5-HT perfusion. It is possible that signals such as CCK and 5-HT, arising from the GI tract, synergistically produce a physiological inhibition of food intake at the level of vagal afferent neurons. Additional evidence supporting this hypothesis is that CCK-induced expression of c-Fos-positive nuclei in select subnuclei of the nucleus tractus solitarius and area postrema is attenuated by a blockade of 5-HT₃ receptors (17), suggesting that 5-HT₃ receptors may directly mediate a select subpopulation of CCK receptive vagal neurons. Indeed, it has repeatedly been established that CCK-induced inhibition of food intake is attenuated by blockade of 5-HT₃ receptors (17, 38, 39). Finally, our group (39) recently reported that sucrose consumption at any dose of ondansetron tested. This is in agreement with our laboratory’s previous work (38, 39) as well as that of others (7, 17) showing that administration of ondansetron in equivalent doses did not alter intake compared with control. Because ondansetron (1.0 mg/kg) does not increase sucrose intake in non-food-deprived rats (39), it is not likely that an orexigenic effect of ondansetron was masked due to a ceiling effect resulting from overnight food deprivation. Furthermore, simultaneous administration of CCK-1 and 5-HT₃ receptor antagonists produces intake greater than that of control in food-deprived rats (39), showing that sucrose intake after overnight deprivation is not at its upper limit per se. Thus it is not likely that our observations result from independent, additive orexigenic and anorexigenic effects of ondansetron and nutrient infusion, respectively, given that ondansetron attenuated the suppression of intake only when rats were infused with higher concentrations of carbohydrate solutions. Instead, our results imply that the feeding effects evoked by 5-HT₃ receptor antagonism are due to inhibition of anorectic signals arising from intestinal carbohydrate-induced activation of 5-HT₃ receptors.

Consistent with the findings of others (53, 62, 63), we found that administration of the competitive α-glucosidase inhibitor, acarbose, attenuated reduction of food intake by intestinal Polyose. This feeding effect of a combined infusion of acarbose and Polyose was maintained when rats were pretreated with ondansetron. We reason that hydrolysis of glucose polymers to the monosaccharide glucose is necessary to elicit reduction of food intake mediated through 5-HT₃ receptors. Supporting this notion is the fact that suppression of intake following duodenal infusion of free luminal glucose was attenuated by blockade of 5-HT₃ receptors. Although the mechanism by which 5-HT is released in response to intestinal glucose remains unclear, there is some evidence that luminal SGLT₁ may be a factor. Specifically, Kim et al. (43), employing an enterochromaffin cell model, demonstrated that glucose stimulates a concentration-dependent release of 5-HT and that this response was inhibited by treatment with phloridzin. In our final experiment, we sought to examine whether active glucose absorption by SGLT₁ was necessary to elicit 5-HT₃ receptors to reduce food intake. Here, duodenal infusion of phloridzin attenuated glucose-induced suppression of intake. This effect was immediate, although transport of glucose into the blood was markedly inhibited to the level of blood glucose following saline infusion. It is conceivable that blocking active glucose absorption with phloridzin attenuated the immediate glucose-induced suppression of intake by impeding SGLT₁-mediated release of 5-HT. Moreover, because 5-HT₃ receptors function as autoreceptors in the regulation of 5-HT release from the small intestine such that activation of the 5-HT₃ receptors triggers a positive feedback mechanism leading to an increase of 5-HT release (23, 54), the attenuation of glucose-induced suppression by ondansetron in our study may have resulted from inhibition of 5-HT release. This notion, along with the inhibitory effect that phloridzin has on 5-HT release (43), could elucidate why ondansetron plus phloridzin had the greatest effect on glucose-
induced suppression of intake. In other words, some but not all glucose-induced 5-HT release was independently inhibited by ondansetron or by phloridzin treatments alone, and this effect was potentiated when these treatments occurred concurrently. Although blockade of 5-HT3 receptors did independently produce a significant inhibition of glucose-induced suppression of intake in the presence or absence of phloridzin, we can only speculate the role of 5-HT release and its activity in our study. However, on the basis of the evidence presented here, we can conclude that intestinally infused glucose suppresses intake partly through a preabsorptive activation of 5-HT3 receptors.

Because 5-HT3 receptors have been shown to be responsive to hyperosmolar liquids to mediate GI functions (46, 49, 61), whenever possible, we chose to infuse solutions that were isosmotic to luminal osmolarity. Although we did not directly measure osmoconcentration of our solutions in the duodenum, it has been shown that infusions of glucose polymer solutions result in lower luminal osmolarity than infusions of glucose solutions of the same concentration by weight (18) and that duodenal infusion of hypertonic glucose solutions produce a maximal duodenal osmoconcentration much lower than that of the solution infused (18, 40). In our study, ondansetron attenuated the suppression induced by 263 mM Polycose as well as 990 mM glucose but not 990 mM mannitol. This suggests that the feeding responses observed when we infused a solution with a high osmotic concentration, or potentially high luminal concentration, were not due to osmotic activation of the 5-HT3 receptors. Additionally, experiment 3 revealed that 1.0 mg/kg ondansetron attenuated glucose-induced suppression of intake to the level of mannitol-induced suppression of intake, suggesting that glucose itself and not simply the colligative properties of the infusate are eliciting activation of 5-HT3 receptors to inhibit food intake.

In conclusion, the present studies demonstrate that 5-HT3 receptors participate in food intake inhibition in response to intraintestinal infusion of both glucose polymer and glucose solutions. We determined that suppression of feeding due to infusion of Polycose as mediated through 5-HT3 receptors requires luminal hydrolysis of the glucose polymer. Likewise, blockade of 5-HT3 receptors resulted in a significant attenuation of suppressed intake when glucose, but not mannitol, was infused at the highest concentration. Ondansetron independently produced a significant inhibition of glucose-induced suppression of intake when luminal glucose absorption was inhibited by phloridzin. Collectively, these findings support the hypothesis that duodenal carbohydrate solutions suppress intake in part through 5-HT3 receptors. Furthermore, 5-HT3 receptor mediation of intestinal Polycose-induced suppression of intake requires hydrolysis but is independent of active glucose transport by luminal SGLT1.

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