Serotonin-type 3 receptors mediate intestinal lipid-induced satiation and Fos-like immunoreactivity in the dorsal hindbrain

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Submitted 2 October 2006; accepted in final form 8 November 2006

IT IS WELL ESTABLISHED THAT nutrients within the duodenal lumen early in a meal play an essential role in producing satiation that occurs with meal ingestion. This is supported by evidence that duodenal infusion of lipids reduce sham food intake (20), whereas intravenous infusion of lipids does not (21), demonstrating that the satiating effect of intraduodenal lipids occurs largely before absorbed lipids enter the blood (19). The predominant hypothesis is that paracrine or endocrine products of intestinal origin, secreted in response to intestinal nutrients, activate vagal afferent fibers leading to inhibition of food intake (11, 16, 42). The indoleamine serotonin (5-HT) is one such signal. Over 95% of the body’s 5-HT is located in the gut, with at least 90% present in enterochromaffin (EC) cells that are scattered in the enteric epithelium from the stomach through the colon (15, 17). The abundance of 5-HT in the gut has made this neuromodulator the focus of numerous studies investigating regulatory feedback mechanisms of food intake.

Systemic serotonergic activity regulates gastrointestinal functions through activation of a number of receptors, including the excitatory, ligand-gated cation channel 5-HT type-3 (5-HT3) receptor. This receptor type is predominantly located on terminals of vagal afferent fibers (8, 18, 43) and is also found within distinct subpopulations of the brain (7, 41). In addition to their role in motility, sensory, and secretory functions (14, 43, 53), 5-HT3 receptors also contribute to physiologic inhibition of food intake in a variety of feeding paradigms (1, 10, 24, 29). For example, studies employing the selective 5-HT3 receptor antagonist ondansetron have revealed that this receptor type mediates suppression of intake following intestinal preloads of carbohydrate solutions (47). Similarly, in a 2002 abstract, Bucinkskaite et al. (5) reported that treatment with ondansetron attenuated the suppression of intake resulting from intestinal infusion of a mixed meal (Ensure). Since 5-HT3-induced suppression of food intake is mediated in part by 5-HT3 receptors (26), and systemic 5-HT activity is associated with reduction of fat intake (3, 49), it is conceivable that 5-HT3 receptors also participate in satiation induced by intraduodenal infusion of lipids. Previously, Burton-Freeman et al. (6) investigated the role of endogenous 5-HT in lipid-induced satiety using tropisetron, a receptor antagonist that exhibits affinity for both 5-HT3 and 5-HT4 receptors (30). The authors reported that tropisetron attenuated the inhibitory effect of an 80% lipid infusion on intermeal interval (6). Furthermore, they showed that simultaneous blockade of cholecystokinin type-1 (CCK1) and 5-HT3/4 receptors reversed the satiating effect of intestinal lipid infusion to a greater extent than antagonism of CCK1 receptors alone. Because tropisetron exhibits affinity for 5-HT4 receptors (30), it remains unclear whether 5-HT3 receptors alone participate in lipid-induced satiation. Therefore, the present study was designed to examine whether inhibition of intake following administration of lipids in the intestine is mediated by 5-HT3 receptors using a potent and highly selective 5-HT3 receptor antagonist (30). Specifically, we assessed both liquid (15% sucrose) and solid (standard chow) food intake in response to intraintestinal infusion of a triglyceride emulsion (Intralipid) when rats were pretreated with ondansetron.

Several investigators have found that intraintestinal lipid, delivered in concentrations effective at decreasing food intake,
activates neurons in the dorsal vagal complex (DVC) as assessed by immunohistochemical detection of increased Fos protein expression (36, 40, 54). This lipid-induced neuronal activation in the nucleus of the solitary tract (NTS), area postrema (AP), and dorsal motor nucleus of the vagus nerve (DMV) occurs largely via vagal afferent activation (36). Given that vagal afferents mediating intraintestinal nutrient and 5-HT signaling terminate in the NTS (33, 51) and that intestinal nutrient-induced satiation requires neuronal processing of DVC structures (52), quantification of Fos-like immunoreactivity (Fos-LI) in the dorsal hindbrain was used as a functional neuroanatomical approach for indexing behavioral anorectic effects of intraintestinal Intralipid. Specifically, we hypothesized that 5-HT3 receptors mediate intraintestinal Intralipid-induced Fos-LI in the DVC of rats.

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 06:00) in a temperature-controlled vivarium. Rats had ad libitum access to pelleted rodent chow (Purina, 5001) and tap 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 (51). Catheters were inserted 2-cm distal to the pylorus and advanced 6-cm within the duodenal lumen in an aborad direction. The exposed 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 time rats attained their preoperative body weights before the experiments began. This protocol was approved by The Pennsylvania State University Institutional Animal Care and Use Committee.

Experiment 1. Effects of 5-HT3 receptor blockade on Intralipid-induced inhibition of 15% sucrose intake. In the first experiment, 15% sucrose (wt/vol) intake was measured (0.1 ml) across 1 h in overnight food-deprived rats (n = 10) following a 20-min duodenal infusion of 72 (0.5 kcal/ml) or 130 mM (1.0 kcal/ml) Intralipid (20%, Baxter Healthcare; VWR International, Bridgeport, NJ). Intestinal Intralipid concentrations were selected based on their efficacy to suppress 60-min intake in food-deprived rats, as determined in pilot studies by our laboratory. Infusates were prepared immediately prior to testing (pH of 7.35–7.40) and delivered using a syringe infusion pump (model 22; Harvard Apparatus, Holliston, MA) 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 (31). Sodium chloride (Sigma, St. Louis, MO) was added to the infusion solutions as needed to yield an isotonic solution (~300 mOsmol). Five minutes prior to intestinal infusion, rats received a 1.0 ml/kg ip injection of the selective 5-HT3 receptor antagonist ondansetron HCl (GlaxoSmithKline, Barnard Castle, UK) across a range of doses (0.125, 0.25, 0.5, 1.0, 2.0, and 5.0 mg/kg) or physiological saline vehicle. Treatments (ondansetron injection and/or nutrient infusion) were separated by saline injection combined with an isotonic (150 mM) saline infusion (Sal/Sal), and each experimental trial was separated by 48 h. A minimum of two repetitions of each injection/infusion combination were conducted for each experiment. Thus, the experimental data are the mean ± SE sucrose from at least two tests separated by Sal/Sal control tests.

Experiment 2. Effects of 5-HT3 receptor blockade on Intralipid-induced inhibition of solid food intake. A separate group of rats (n = 7) were adapted to consuming standard rodent chow placed on the floor of their home cage. As in experiment 1, following an overnight food deprivation, rats were injected with saline or ondansetron (0.125, 0.25, 0.5, 1.0, 2.0, and 5.0 mg/kg) 5 min before the initiation of an intraintestinal infusion of saline or Intralipid. Preliminary testing revealed that 72 mM Intralipid did not significantly suppress chow intake beyond 30 min (data not shown). Therefore, only 130 mM Intralipid was infused in this experiment. After infusion ceased, rats were immediately returned to their home cages and a preweighed amount of standard rodent chow was placed on the cage floor. Food intake was recorded (0.1 g) at 0.5, 1, 2, and 4 h following presentation, taking into account spillage that was collected in a tray placed under the cage. Treatments were administered as described in experiment 1.

Experiment 3. Effects of 5-HT3 receptor blockade on Intralipid-induced Fos-LI in the DVC. Rats previously used in infusion experiments (n = 32) were divided into groups of eight for the immunohistochemical detection of Fos. Fos-LI in the dorsal hindbrain of rats in each group was examined 90 min after an injection of saline or ondansetron (1.0 mg/kg ip) followed by intraintestinal infusion of 130 mM Intralipid or saline. Thus, four experimental groups were established as follows: 1) saline injection accompanied by saline infusion (Sal/Sal), 2) ondansetron injection accompanied by saline infusion (Ond/Sal), 3) saline injection accompanied by Intralipid infusion (Sal/Int), and 4) ondansetron injection accompanied by Intralipid infusion (Ond/Int).

The experimental protocol was identical to that used in the feeding studies described above with the exception that upon completion of the intestinal infusion, rats were returned to their home cages without access to food or water until the time of death. Thirty-five minutes after the infusion ceased, the rats were deeply anesthetized [ketamine (50 mg/kg), xylazine (5 mg/kg), and acepromazine (1 mg/kg) mixture; 1 ml/kg] and transcardially perfused with 0.1 M sodium phosphate buffer, pH 7.4, followed by 4% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.4. Brains were removed and postfixed for 4 h in 4% formaldehyde. The brains were subsequently stored in 20% sucrose overnight to cryoprotect them and reduce freezing artifact. Thirty-micrometer coronal sections were cut from the hindbrain using a cryostat (Leica Microsystems). The sections were incubated for 24 h at room temperature in primary antiserum (1:50,000) raised in rabbit against a synthetic peptide representing amino acids 4–17 of human Fos (Ab-5; EMD Biosciences, La Jolla, CA). After washing and subsequent overnight incubation in a biotinylated donkey anti-rabbit serum (1:500; Jackson Immuno-Research Laboratories, West Grove, PA), sections were incubated in avidin conjugated to horseradish peroxidase. The sections were subsequently washed and processed to reveal horseradish peroxidase activity using diaminobenzidine intensified with nickel as previously described by Ritter and Dinh (46). Dehydrated, cleared sections were mounted and coverslipped, and quantification of Fos immunoreactive nuclei was performed. An observer, who was blind to the experimental treatments, counted bilateral Fos-LI nuclei twice, at four different coronal brain levels (~13.24, ~13.30, ~13.80, and ~14.60 mm caudal to bregma) according to the stereotaxic atlas of Paxinos and Watson (39). A minimum of three sections per brain level were analyzed for each rat. Using bright-field microscopy, cells with black nuclear Fos staining, which was easily recognized against the generally low background, were identified as positive cells. Fos positive cells within the nuclei composing the DVC (NTS, AP, and the DMV) were counted. The presented data are the means ± SE number of Fos-LI neurons at each brain level as calculated for each rat and treatment condition.

Statistical analyses. Cumulative 15% sucrose solution and chow intakes for each time point were analyzed by two-way repeated-measures ANOVA (rmANOVA), with injections and intestinal infusions as independent variables, using PC-SAS (version 8.02; SAS Institute, Carey, NC) mixed procedure. Differences in the numbers of
**Table 1. Effects of ondansetron on 72 mM Intralipid-induced inhibition of sucrose intake**

<table>
<thead>
<tr>
<th>Injection</th>
<th>Infusion</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Saline</td>
<td>15.6±0.5</td>
<td>16.0±0.5</td>
</tr>
<tr>
<td>Saline</td>
<td>72 mM Int</td>
<td>9.2±0.7*</td>
<td>11.1±0.6*</td>
</tr>
<tr>
<td>0.125 mg/kg Ond</td>
<td>72 mM Int</td>
<td>9.8±0.8*</td>
<td>11.6±0.8*</td>
</tr>
<tr>
<td>0.25 mg/kg Ond</td>
<td>72 mM Int</td>
<td>9.8±0.9*</td>
<td>12.3±0.8*</td>
</tr>
<tr>
<td>0.5 mg/kg Ond</td>
<td>72 mM Int</td>
<td>11.5±0.9†</td>
<td>12.6±0.9†</td>
</tr>
<tr>
<td>1.0 mg/kg Ond</td>
<td>72 mM Int</td>
<td>12.2±0.6†</td>
<td>13.4±0.5†</td>
</tr>
<tr>
<td>2.0 mg/kg Ond</td>
<td>72 mM Int</td>
<td>12.5±0.9†</td>
<td>13.9±0.9†</td>
</tr>
<tr>
<td>5.0 mg/kg Ond</td>
<td>72 mM Int</td>
<td>12.4±1.0†</td>
<td>13.5±1.0†</td>
</tr>
</tbody>
</table>

Values are means ± SE 15% sucrose intakes (ml) in overnight food-deprived rats (n = 10). Infusion with 72 mM Intralipid (Int) produced a significant reduction of 30- and 60-min 15% sucrose intake. Ondansetron (Ond) administered at 0.5, 1.0, 2.0, and 5.0 mg/kg each significantly attenuated 72 mM Intralipid-induced suppression of intake at both time points. *Significantly different from intake following saline injection/saline infusion for each time point (P < 0.05).

**RESULTS**

In all experiments, when rats received ondansetron prior to intestinal saline infusion, intake and Fos-LI were not significantly different from control (saline injection/saline infusion) at any time (all P > 0.05). Intakes following the bracketed control or Intralipid treatments did not significantly change across tests (all P > 0.05).

**Intralipid-induced inhibition of 15% sucrose intake following 5-HT₃ receptor blockade.** The effects of treatment with ondansetron on Intralipid-induced inhibition of 30- and 60-min sucrose intakes are presented in Tables 1 and 2. At 30 min, significant main effects of ondansetron injection [F(6,319) = 10.93; P < 0.001] and Intralipid infusion [F(2,319) = 154.86; P < 0.001] were revealed by two-way rmANOVA. There was also a significant interaction between ondansetron and Intralipid [F(6,319) = 5.01; P < 0.001] evident at this time point. At 60 min, results of two-way rmANOVA revealed a significant effect of ondansetron injection [F(6,319) = 11.55; P < 0.001], Intralipid infusion [F(2,319) = 199.12; P < 0.001], as well as a significant interaction between ondansetron and Intralipid [F(6,319) = 5.79; P < 0.001]. Infusion with 72 mM Intralipid produced a significant reduction of 30- and 60-min 15% sucrose intake (P <0.001 for both time points). Ondansetron administered at 0.5, 1.0, 2.0, and 5.0 mg/kg, each significantly attenuated 72 mM Intralipid-induced suppression of intake at both time points (P < 0.05 for each dose at both time points), whereas lower doses (0.125 or 0.25 mg/kg) did not. Similarly, duodenal infusion with 130 mM Intralipid produced a significant suppression of 30- and 60-min 15% sucrose intake (P <0.001 for both time points), which was significantly attenuated by pretreatment of 1.0, 2.0, and 5.0 mg/kg ondansetron (P < 0.05 for each dose at both time points). Conversely, 0.125, 0.25, or 0.5 mg/kg ondansetron did not significantly attenuate this suppression at either time point.

**Intralipid-induced inhibition of solid food intake following 5-HT₃ receptor blockade.** The effects of ondansetron pretreatment (0.125–5.0 mg/kg) on Intralipid-induced inhibition of cumulative 30-, 60-, 120-, and 240-min chow intake are presented in Fig. 1. Results of two-way rmANOVA demonstrated a significant effect of Intralipid infusion [F(1,89) = 157.06; P < 0.001] but not a significant main effect of ondansetron [F(6,89) = 1.12; P > 0.4] at 30 min. A significant interaction between ondansetron and Intralipid was also observed at this time point [F(6,89) = 2.78; P < 0.02]. Likewise, at 60 min there was a significant main effect of Intralipid infusion [F(1,89) = 28.35; P < 0.001] and a significant effect of ondansetron [F(6,89) = 3.38; P < 0.04], as well as a significant interaction between ondansetron and Intralipid [F(6,89) = 2.13; P < 0.05]. At 120 min, there was a significant main effect of Intralipid infusion [F(1,89) = 11.92; P < 0.001] and a significant effect of ondansetron [F(6,89) = 2.72; P < 0.02], but not a significant interaction between ondansetron and Intralipid [F(6,89) = 1.56; P > 0.1]. At 240 min, two-way rmANOVA revealed a significant main effect of Intralipid infusion [F(1,89) = 10.8; P < 0.001] and a significant effect of ondansetron [F(6,89) = 2.87; P < 0.02], but not a significant interaction between ondansetron and Intralipid [F(6,89) = 1.58; P > 0.1].

Infusion with 130 mM Intralipid produced a significant reduction of chow intake (P < 0.001) at each respective time point. Ondansetron administered at 0.5 mg/kg significantly attenuated Intralipid-induced suppression of intake at 60 and 120 min (P < 0.05). Suppressed intake was also significantly attenuated by higher doses (1.0, 2.0, and 5.0 mg/kg) at the end of 60 min (P < 0.05) and significantly reversed at 120 min (P < 0.01). These effective doses of ondansetron (0.5–5.0 mg/kg) all significantly reversed the suppression of intake at the end of 240 min (P < 0.05). Conversely, 0.125 or 0.25 mg/kg ondansetron did not significantly attenuate the 130 mM Intralipid-induced suppression of chow intake at any time point.

**Effects of 5-HT₃ receptor blockade on Intralipid-induced Fos-LI in the DVC.** A significant effect of experimental treatment on DVC Fos-LI was demonstrated by two-way ANOVA [F(3, 28) = 23.85; P < 0.0001]. There was also a significant

**Table 2. Effects of ondansetron on 130 mM Intralipid-induced inhibition of sucrose intake**

<table>
<thead>
<tr>
<th>Injection</th>
<th>Infusion</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Saline</td>
<td>14.2±0.4</td>
<td>15.5±0.5</td>
</tr>
<tr>
<td>Saline</td>
<td>130 mM Int</td>
<td>2.8±0.4*</td>
<td>4.3±0.5*</td>
</tr>
<tr>
<td>0.125 mg/kg Ond</td>
<td>130 mM Int</td>
<td>2.5±0.5*</td>
<td>3.9±0.8*</td>
</tr>
<tr>
<td>0.25 mg/kg Ond</td>
<td>130 mM Int</td>
<td>3.6±0.4*</td>
<td>5.0±0.6*</td>
</tr>
<tr>
<td>0.5 mg/kg Ond</td>
<td>130 mM Int</td>
<td>3.5±0.5*</td>
<td>4.3±0.5*</td>
</tr>
<tr>
<td>1.0 mg/kg Ond</td>
<td>130 mM Int</td>
<td>6.6±0.8†</td>
<td>7.9±0.5†</td>
</tr>
<tr>
<td>2.0 mg/kg Ond</td>
<td>130 mM Int</td>
<td>7.0±0.7†</td>
<td>8.8±0.8†</td>
</tr>
<tr>
<td>5.0 mg/kg Ond</td>
<td>130 mM Int</td>
<td>7.2±0.8†</td>
<td>8.9±0.6†</td>
</tr>
</tbody>
</table>

Values are means ± SE 15% sucrose intakes (ml) in overnight food-deprived rats (n = 10). Infusion with 130 mM Int produced a significant reduction of 30 and 60 min 15% sucrose intake. Ond administered at 1.0, 2.0, and 5.0 mg/kg each significantly attenuated 130 mM Int-induced suppression of intake at both time points. *Significantly different from intake following saline injection/saline infusion for each time point (P < 0.05). †Significantly different from intake following saline injection/Int infusion for each time point (P < 0.05).
interaction between treatment and brain levels examined \( F(9, 868) = 50.06; P < 0.0001 \). Following ondansetron treatment, Fos-LI in rats infused with saline (Ond/Sal) was not significantly different from control (Sal/Sal). However, intraintestinal infusion of Intralipid (Sal/Int) induced an increase in Fos-LI at all levels of the DVC counted \( (P < 0.05) \). Pretreatment with ondansetron (Ond/Int) attenuated the intralipid-induced increase in two of the four brain levels examined \((-13.80 \text{ mm and } -13.30 \text{ mm, caudal to bregma}; P < 0.001)\). The mean counts of Fos-LI by treatment group within the nuclei composing the DVC (subnuclei of the NTS, AP, and the DMV) are presented in Fig. 2.

**DISCUSSION**

Our findings demonstrate that pretreatment with a highly selective 5-HT\textsubscript{3} receptor antagonist attenuates suppression of both liquid (15% sucrose) and solid (rodent chow) food intake resulting from duodenal infusion of Intralipid. In addition to the behavioral effects on food intake, this study also demonstrates that 5-HT\textsubscript{3} receptors mediate dorsal hindbrain neuronal...
activation since pretreatment with ondansetron reduced Intralipid-induced Fos-LI. These results provide the first direct evidence that 5-HT$_3$ receptors participate in lipid-induced satiation and are consistent with previous findings demonstrating that 5-HT$_3$ receptors mediate suppression of intake in response to intestinal preloads, including infusions of a complete liquid meal (Ensure) (5), as well as carbohydrate solutions (47).

Although the mechanisms by which 5-HT$_3$ receptors participate in suppression of food intake by nutrients are not completely elucidated, 5-HT release in response to luminal stimulation represents an important signal. The neurophysiological effects of 5-HT in the gut are mediated through several classes of 5-HT$_3$ receptors, including 5-HT$_3$ receptors that are involved in control of food intake and various GI functions including, but not limited to peristalsis, digestive secretions, nociceptive and emetic sensation, as well as gastric emptying (14, 43, 53). Little is known, however, about the involvement of 5-HT in intestinal lipid detection and signal transduction. To our knowledge, there is no direct evidence demonstrating 5-HT release in response to the presence of fats in the gastrointestinal tract. Most of the available evidence indicates that luminal factors, such as osmolarity, the products of carbohydrate digestion, and mucosal mechanical stimulation, all elicit 5-HT release from EC cells (32, 34, 38, 53). However, it is conceivable that mucosal stimulation by lipid infusate may trigger 5-HT release from EC cells into the lamina propria. It is known that 5-HT is released in response to luminal pressure, and it could be that this source of 5-HT is sufficient to act on vagal terminals in a paracrine fashion to elicit an effect. In addition, intraintestinal lipid infusion produces inhibition of gastric emptying (31) and subsequent gastric distention. Because gastric distention stimulates 5-HT release (35), an alternative source of 5-HT activating 5-HT$_3$ receptors in this study might originate from the gastric EC cells. Glatzle et al. (18) have shown that 5-HT$_3$ receptors are found along the length of the GI tract, myenteric plexus, smooth muscles, and mucosa. Also within the duodenum, 5-HT$_3$ receptors are present on vagal afferent terminals, and in the guinea pig, at least, 5-HT$_3$ receptors are involved in mediating activation of intrinsic primary afferent neurons from the mucosa (2). These neurons are activated by mucosal stimulation following mechanical stimuli, short-chain fatty acids, or 5-HT application (14, 17). It is clear that 5-HT is capable of producing changes in activity of vagal primary afferent neurons terminating in the solitary nucleus of the medulla. Electrophysiological recordings of vagal afferents innervating the small intestine are extremely sensitive to exogenous, as well as endogenous 5-HT released in response to luminal nutrients, a response mediated via 5-HT$_3$ receptors (22, 33). In vivo investigations also demonstrate that endogenously released 5-HT plays a major role in signal transmission evoked by luminal nutrients to stimulate vagal fibers via 5-HT$_3$ receptor activation (35). Therefore, given the immunohistochemical and electrophysiological data, our results provide indirect evidence that Intralipid, may activate 5-HT$_3$ receptors on vagal afferents through release of 5-HT either directly (i.e., mucosal stimulation) or via gastric distention.

While our results show that selective blockade of 5-HT$_3$ receptors attenuate lipid-induced suppression of intake of both solid and liquid foods, the lowest effective dose of ondansetron differed slightly with regard to the concentration of lipid and the type of food being tested. For example, 0.5 mg/kg ondansetron was the lowest effective dose to attenuate suppression of intake when rats were infused with 72 mM Intralipid and tested on sucrose, as well as when rats were infused with 130 mM Intralipid and tested on chow. However, when sucrose intake was measured in rats infused with 130 mM Intralipid, 1.0 mg/kg ondansetron was the lowest effective dose to attenuate suppression of intake. The difference between the two experiments might be due to the degree by which intake is suppressed in conjunction with the type of test meal. For example 72 mM Intralipid caused ~34% suppression of 60-min sucrose or chow intake, whereas the percent suppression of sucrose intake by 130 mM Intralipid was ~68%. Thus it appears that the effectiveness of ondansetron to attenuate Intralipid-induced reduction of intake depends on the strength of the inhibition signal. That is, a lower dose (0.5 mg/kg) of ondansetron is sufficient to attenuate a relatively small degree of intake suppression, whereas higher doses (1.0–5.0 mg/kg) are necessary to attenuate a greater degree of suppression. These results are consistent with our previous findings showing that 1.0 mg/kg was the lowest effective dose of ondansetron to attenuate a relatively larger degree of suppression resulting from duodenal carbohydrate solutions (47). Another possible expla-
nation for the differing degree of 5-HT3 participation in mediating Intralipid-induced satiation would involve the putative varying degree of gastric feedback between the two Intralipid concentrations. Gastric feedback plays an important role in 5-HT3 receptor mediation of CCK-induced suppression of food intake (25, 28). Therefore, as our results indicate, a more concentrated intestinal lipid load induces a greater suppression of intake, presumably involving cholecystokininergic signaling, inhibition of gastric emptying, and prolonged retention of gastric contents. The temporal increase in gastric distension leads to 5-HT release and activation of 5-HT3 receptors (25, 35). Thus, it is not surprising that a higher dose of ondansetron is required to attenuate suppression of intake in response to a combined increase in gastric and intestinal feedback by a greater concentration of duodenal Intralipid.

Consistent with previous studies, administration of ondansetron alone did not alter food intake compared with control (10, 28, 29). Recently we reported that systemic ondansetron delivered at doses as high as 5.0 mg/kg does not increase 15% sucrose intake in overnight food-deprived rats (47). Here we replicated and extended these results to intake of solid food. These findings suggest that meal termination of sucrose or chow may occur by subthreshold involvement of 5-HT3 receptor-mediated signaling in conjunction with other anorectic signals. This would certainly fit with our previous data showing that ondansetron attenuates satiation by relatively high concentrations of intestinal carbohydrates (47) exogenous CCK (29), and gastric distension (25). Therefore, given that ondansetron alone did not increase intake, it is likely that our observations did not result from independent, additive or synergistic effects of ondansetron and lipid infusion, respectively. Instead, our findings suggest that the feeding effects evoked by 5-HT3 receptor antagonism are due to inhibition of anorectic signals arising from intestinal Intralipid-induced activation of 5-HT3 receptors. While systemic administration of ondansetron does not increase meal size, we have recently shown that direct parenchymal administration of ondansetron in the NTS increased sucrose intake (27). Thus, there may well be divergence of 5-HT3 receptor-mediated control of meal size, with different and anatomically distinct receptor populations, mediating separate and possibly overlapping aspects of gastrointestinal-derived anorectic signals.

Although the findings presented here demonstrate that 5-HT3 receptors contribute to lipid-induced reduction of short-term food intake, they cannot account for it entirely. As previously mentioned, it is well documented that one mechanism by which lipids inhibit food intake involves activation of CCK1 receptors through the release of CCK (4, 12, 23, 45). While not directly tested, it has been hypothesized that CCK may act as a secretagogue for 5-HT release (9). Furthermore, simultaneous blockade of CCK1 and 5-HT3 receptors completely reverses the satiating effects of intestinal lipid infusion beyond that of CCK1 receptor blockade alone (6). Therefore, it is likely that lipid-induced satiation may occur via cooperative activation of CCK1 and 5-HT3 receptors by endogenously released CCK and 5-HT. In fact, our laboratory has shown that pretreatment with ondansetron significantly attenuated CCK-induced reductions of food intake when rats are tested on either a 15% sucrose solution (29) or solid maintenance diet (28). Furthermore, systemic CCK and 5-HT act synergistically to suppress food intake, an effect mediated by cooperation between CCK1 and 5-HT3 receptors (26). Therefore, it is likely that the suppression of intake by Intralipid in the current study is due to cooperative afferent signaling by endogenously secreted CCK and 5-HT activating their respective receptors.

Corroborating earlier findings (36, 40, 54), we showed that intraintestinal infusion of Intralipid induced an increase in Fos-LI in the DVC at all levels of the dorsal medulla examined. Intralipid-induced Fos-LI was greater in the NTS with comparison to Intralipid-induced Fos-LI in the DMV and AP. This effect was attenuated in ondansetron-treated rats at the levels of the AP and the caudal fourth ventricle (−13.80 and −13.30 mm from bregma). At these levels, ondansetron treatment reduced lipid-induced Fos-LI within the NTS by ~34% and within the AP by 60%. While Intralipid-induced Fos-LI is not completely blocked by ondansetron treatment, 5-HT3 receptors do appear to be appreciably involved in the vagal signal transmission evoked by luminal nutrients. Several luminal factors, such as osmolarity and the digestion products of carbohydrates activate vagal primary afferent neurons via 5-HT3 receptors. In fact, Wu et al. (50) recently reported that Fos-LI in nodose ganglia neurons resulting from intraintestinal 5-HT or maltose infusion was significantly reduced by administration of a selective 5-HT3 receptor antagonist. We and others have also shown that both gastric distension- and CCK-induced Fos-LI in select subnuclei of the DVC is attenuated by blockade of 5-HT3 receptors (10, 25, 35). It is known that, intraduodenal infusion of lipid elevates circulating CCK levels and induces Fos-LI in a pattern similar to CCK (36). This action is presumably the result of paracrine or endocrine CCK stimulation of vagal afferents or from direct actions of circulating CCK on the AP. Therefore, it is plausible that ondansetron is inhibiting induction of Fos-LI by acting on CCK-receptive neurons.

Our investigation does not clearly distinguish whether ondansetron is acting at peripheral or central 5-HT3 receptors to attenuate lipid-induced suppression of intake or neuronal activation. While much of the available evidence suggests that 5-HT3 receptors mediate gut vagal afferent signaling (34, 50, 53), several other studies reveal that 5-HT3 receptors are widely distributed in areas of the central nervous system related to control of food intake, such as the dorsal hindbrain, most notably in the NTS and AP (37, 41, 44). In fact, previous data from our laboratory reveals that administration of ondansetron into the fourth ventricle attenuates CCK-induced suppression of food intake (27). While penetration of the blood-brain barrier by systemic ondansetron appears to be limited (13, 48), hindbrain regions including the AP, as well as the median eminence have been identified as circumventricular organs (13), allowing a greater degree of permeability of circulating substrates. Therefore, while the exact anatomical site of ondansetron’s effects on Intralipid-induced suppression of intake and neuronal activation cannot be clearly discerned, participation of both peripheral and central 5-HT3 receptors is likely.

In conclusion, we have shown that duodenal Intralipid-induced suppression of liquid sucrose and solid chow intake is attenuated by selective blockade of 5-HT3 receptors. We have also shown that Intralipid-induced Fos-LI in the DVC is attenuated by blockade of 5-HT3 receptors. Collectively, these studies support the hypothesis that 5-HT3 receptors mediate intestinal lipid-induced satiation. In conjunction with our pre-
vious studies, it is possible that participation of 5-HT₃ receptors in lipid-induced suppression of intake involves cooperation with other anorectic systems, such as those involving CCK.

ACKNOWLEDGMENTS

The authors thank Chun-Yi Hung, Bart C. De Jonghe, Mackenzie Tomazin, and Melissa G. Carelle for help conducting these studies. We acknowledge GlaxoSmithKline for their generous donation of ondansetron.

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