

CCK and 5-HT act synergistically to suppress food intake through simultaneous activation of CCK-1 and 5-HT3 receptors

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Received 16 February 2005; received in revised form 18 March 2005; accepted 21 March 2005
Available online 19 April 2005

Abstract

Cholecystokinin (CCK) and serotonin (5-HT) systems have been shown to cooperate interdependently in control of food intake. To assess mechanisms by which CCK and 5-HT systems interact in control of food intake we examined: (1) participation of CCK-1 and 5-HT3 receptors in 5-HT-induced suppression of sucrose intake; (2) the interaction between CCK and 5-HT in suppression of food intake; (3) the role of CCK-1 and 5-HT3 receptors in mediating this interaction. Intraperitoneal administration of 5-HT (0.25, 0.5 and 1.0 mg/kg) significantly reduced intake compared to control in a dose responsive fashion ($r^2 = 0.989$). Suppression of food intake by 5-HT was significantly attenuated by prior treatment with the 5-HT3 receptor antagonist ondansetron at each 5-HT dose tested ($P < 0.05$), while blockade of CCK-1 receptors by lorglumide had no effect on 5-HT-induced suppression of intake. Administration of CCK-8 (0.5 $\mu\text{g}/\text{kg}$) or 5-HT (0.5 mg/kg) alone significantly reduced sucrose intake by 22.9 and 22.2% respectively, compared to control ($P < 0.0001$). Co-administration of CCK and 5-HT resulted in a synergistic suppression of intake leading to an overall 48.4% reduction in sucrose intake compared to saline ($P < 0.0001$). Concomitant CCK-1 and 5-HT3 receptor blockade by lorglumide and ondansetron respectively, resulted in a complete reversal of the combined CCK and 5-HT-induced suppression of intake. Independent administration of lorglumide or ondansetron did not alter intake compared to control. These studies provide evidence that 5-HT causes suppression in food intake by acting at 5-HT3, not CCK-1 receptors. Furthermore, CCK and 5-HT interact to produce an enhanced suppression of food intake, an effect mediated through concomitant activation of CCK-1 and 5-HT3 receptors. © 2005 Elsevier Inc. All rights reserved.

Keywords: Satiety; Gastric; Synergistic

1. Introduction

A number of within-meal gut peptides and neurotransmitters which participate in meal termination are released in response to nutrients entering the duodenum. These signals do not act alone, but instead comprise complementary elements of an integrated system. Cholecystokinin (CCK) and serotonin (5-hydroxytryptamine, 5-HT) are two such humoral signals that exert control on food intake [5,7,24,31,34,48,60]. While independently, both cholecystokinergic and serotoner-

gic systems reduce food intake, they have also been shown to interact in control of food intake and various neural modulatory functions [9,10,21,30,49]. Original investigations suggested that peripheral cholecystokinergic activity recruited the central serotonergic system to reduce meal size (for review see [9,10]), however evidence has also revealed an important role of systemic serotonergic activity in the interaction with the cholecystokinergic system to reduce food intake [7,19,13,41]. Of considerable interest in this regard, is recent data supporting a role for serotonin type-3 (5-HT3) receptors in the modulation of CCK-induced satiation. 5-HT3 receptors have been shown to be located on terminals of vagal afferent fibers innervating the

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gastrointestinal tract [8,22,43], and exhibit vagal excitation in response to various stimuli including but not limited to gastric distension [3,33], intrainestinal nutrients [43], systemic 5-HT and 5-HT-like agonists [22,30], as well as CCK [11,49].

Systemic serotonergic activity induces a suppression of food intake through activation of a number of serotonergic receptors [1,14,18,41,53]. Initial investigation of this phenomenon using indirect serotonergic agonists such as the 5-HT reuptake inhibitor/5-HT releaser *D*-fenfluramine often discounted the role of 5-HT₃ receptors in satiation [26,38], however *D*-fenfluramine and 5-HT have distinct differences in their control of food intake and feeding-related physiological processes [10,14,16,17,36]. Moreover, the satiating effect of *D*-fenfluramine has in part been attributed to its role in macronutrient preference/selection [23,27,56], or as a result of reducing ingestive motor function [15]. Nonetheless, more recent evidence supporting the hypothesis that 5-HT₃ receptors are in fact involved in the feedback control of intake comes from studies showing that selective blockade of 5-HT₃ receptors attenuates nutrient-induced satiation [6,7,50,51], as well as CCK-induced suppression of both solid and liquid food intake [11,19,20]. While 5-HT₃ receptor participation in control of food intake, and the inhibition of food intake by systemic administration of 5-HT is evident, the role of 5-HT₃ receptors in mediating exogenous 5-HT-induced suppression of food intake has not been directly tested.

Considering the fact that independent blockade of either CCK-1 [46,48] or 5-HT₃ [11,19] receptors attenuates CCK-induced satiation, this suggests that CCK-1 and 5-HT₃ receptors are interrelated in control of food intake. Previous findings from our laboratory demonstrating that concomitant blockade of CCK-1 and 5-HT₃ receptors synergistically increases food intake compared to intake following blockade of either receptor alone support this notion [20]. Similarly, Burton-Freeman et al. [7] have shown that CCK-1 and 5-HT₃ receptors act in concert to mediate intrainestinal nutrient-induced suppression of food intake. While these findings indicate that endogenous CCK and 5-HT may interact at these specific receptors to reduce intake, to date there has been no direct examination of the type and extent of interaction between systemic CCK and 5-HT. Furthermore, the contribution of CCK-1 and 5-HT₃ receptors, either alone or in association, in mediating the feeding effects in response to simultaneous action of exogenous CCK and 5-HT has not been investigated.

Therefore, using selective CCK-1 and 5-HT₃ receptor antagonists, we tested the hypothesis that systemic administration of 5-HT reduces intake through 5-HT₃, not CCK-1 receptor activation. Additionally, we hypothesized that anorectic signals produced by co-administration of 5-HT and CCK are integrated and amplified, resulting in an enhanced suppression of food intake. Finally, we examined whether the enhanced suppression of intake following concomitant administration of CCK and 5-HT is due to simultaneous CCK-1 and 5-HT₃ receptor activation.

2. Methods

2.1. *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 h:12 h light–dark cycle (lights off at 1800 h). Rats had ad libitum access to standard rat chow (Purina, 5001) and water except as indicated in the next section when they were deprived of food but not water overnight (16 h). Prior to testing, animals were adapted to experimental conditions for 1 week. This protocol was approved by The Pennsylvania State University Institutional Animal Care and Use Committee.

The drugs used in these experiments were cholecystokinin octapeptide sulfate (CCK-8; American Peptide Inc., Sunnyvale, CA), serotonin creatinine sulfate complex (5-HT; Sigma, St. Louis, MO) ondansetron (2 mg/ml, Burns Veterinary Supply, Rockville, NY), a selective 5-HT₃ receptor antagonist, and lorglumide (Sigma, St. Louis, MO), a selective CCK-1 receptor antagonist. All drugs were dissolved in sterile 0.9% saline, and were administered via an intraperitoneal (IP) injection in a volume of 1.0 ml/kg body weight.

2.2. *Experimental procedure*

During testing, water was removed 45 min prior to presentation of 15% sucrose solution. At 0800 h each animal received one or two injections of either saline or drug, as detailed below. Five minutes following drug administration, rats were returned to their homecage and presented with a calibrated tube containing 15% (w/v) sucrose solution. Intake was measured to the nearest 0.1 ml every 5 min over the ensuing 1 h. A minimum of 48 h elapsed between each experimental trial. For experiments 1 and 2, all rats received the same drug treatment on a given test day with each trial being separated by a saline injection. For experiment 3, drug conditions were administered in a pseudo-randomized fashion, where all drug conditions were administered on every test day with each rat receiving all possible drug conditions at least twice.

2.3. *Experiment 1: 5-HT-induced suppression of food intake following blockade of 5-HT₃ receptors*

In a preliminary dose response experiment, we examined the effects of 5-HT₃ receptor blockade by ondansetron on food intake in food deprived animals. Rats ($n = 8$) fasted for 16 h received an IP injection of saline or ondansetron (0.125, 0.25, 0.5, or 1.0 mg/kg) five min before sucrose presentation. Food intake was recorded every 5 min for the ensuing 1 h. To determine if 5-HT₃ receptors mediate systemic 5-HT-induced suppression of food intake, in a separate experiment, ondansetron was administered and tested for its effectiveness to reverse 5-HT-induced suppression of intake. During testing, rats received one of the following drug

conditions 5 min before sucrose presentation: saline, ondansetron (1.0 mg/kg), 5-HT (0.25, 0.5, or 1.0 mg/kg), or a combined injection of ondansetron (1.0 mg/kg) and 5-HT (0.25, 0.5, or 1.0 mg/kg). The 1.0 mg/kg dose of ondansetron was chosen based on results from the ondansetron dose-response experiment and existing literature demonstrating that 1.0 mg/kg of ondansetron is effective in attenuating both CCK- and intraintestinal nutrient-induced suppression of intake [11,19,20,51].

2.4. Experiment 2: 5-HT-induced suppression of food intake following blockade of CCK-1 receptors

Using the same group of rats ($n = 8$) from the previous experiment, we tested the ability of the CCK-1 receptor blocker lorglumide, to attenuate 5-HT-induced suppression of intake. In brief, overnight food deprived rats received an IP administration of saline or lorglumide (1.0 mg/kg) 15 min prior to an injection of saline or 5-HT (1.0 mg/kg). Sucrose was presented 5 min following the second drug injection. The 1.0 mg/kg dose of 5-HT was chosen based on the dose response study from the previous experiment, as this was the only dose not completely reversed by ondansetron, while the 1.0 mg/kg dose of lorglumide was chosen based on previous reports demonstrating its effectiveness in blocking CCK-1 receptors leading to a reversal of CCKs effects on food intake and other gastrointestinal functions [20,60].

2.5. Experiment 3: CCK-1 and 5-HT₃ receptor participation in suppression of food intake by simultaneous administration of CCK and 5-HT

2.5.1. Experiment 3a

This study examined feeding responses following simultaneous systemic administration of CCK and 5-HT. After an overnight fast, a separate group of rats ($n = 16$) received an injection containing either saline, CCK (0.05 or 0.5 $\mu\text{g}/\text{kg}$), 5-HT (0.1 or 0.5 mg/kg), or a combined injection of CCK and 5-HT. Two drug combination doses were chosen based on their effectiveness to inhibit intake: (1) smaller subthreshold doses which had no effect on food intake when given independently (CCK: 0.05 $\mu\text{g}/\text{kg}$; 5-HT: 0.1 mg/kg), and (2) higher doses which by themselves produced a significant suppression in food intake (CCK: 0.5 $\mu\text{g}/\text{kg}$; 5-HT: 0.5 mg/kg). Sucrose was presented 5 min following drug administration.

2.5.2. Experiment 3b

To determine if a relationship exists between CCK-1 and 5-HT₃ receptors in control of CCK plus 5-HT-induced suppression of food intake, both receptor antagonists (lorglumide and ondansetron, respectively) were administered and tested for their effectiveness to reverse the satiating effects produced by concomitant administration of CCK and 5-HT. Briefly, overnight food deprived rats used in the previous experiment received an injection containing either saline, lorglumide (1.0 mg/kg), ondansetron (1.0 mg/kg), or a combined

injection of lorglumide and ondansetron followed 15 min later by an injection of either saline, or a combined single injection of CCK (0.5 $\mu\text{g}/\text{kg}$) and 5-HT (0.5 mg/kg). Sucrose was presented five min following the second drug injection.

2.6. Data and statistical analyses

Data for each respective study were analyzed separately and expressed as mean \pm S.E.M. Sucrose solution (15%) intakes for all time points were analyzed by repeated measures analysis of variance (rmANOVA), with drug treatments as the main variables. For all experiments, significant differences among treatment means (adjusted) were analyzed by pairwise *t*-test for planned comparisons with $P < 0.05$ considered statistically significant. Data from 5-HT dose response experiment were analyzed by linear regression (r^2). All analyses were made using PC-SAS (version 8.02, SAS Institute, Cary, NC) mixed procedure. To determine the nature of the interaction between CCK and 5-HT when simultaneously administered, the Bliss Independence Model [12] was applied. Briefly, this equation estimates the additive response to a combination of two drugs (e.g., A and B) equals the fractional response of drug A (F_A) added to the fractional response of drug B (F_B) multiplied by the remaining possible response ($1 - F_A$): $F_{AB} = F_A + F_B (1 - F_A)$. The assumption for using the Bliss Independence Model is that both drugs must act on the same system (at least down stream) such that the maximum response provoked by each agent is the same [2,12].

3. Results

3.1. Experiment 1: 5-HT-induced suppression of food intake following blockade of 5-HT₃ receptors

One-way rmANOVA revealed no significant effect of ondansetron treatment on food intake at 30 min [$F(4, 36) = 0.63$; $P = 0.645$] or 60 min [$F(4, 36) = 0.80$; $P = 0.534$] at any dose (0.125, 0.25, 0.5, or 1.0 mg/kg) tested. When analyzing the effect of 1.0 mg/kg dose of ondansetron on 5-HT-induced suppression of intake, there was an overall significant main effect of drug treatment [$F(7, 85) = 6.01$; $P < 0.0001$]. Given that exogenous 5-HT has been shown to reduce intake during an animal's first meal following administration [28], we choose to report the initial contribution of 5-HT₃ receptors in mediating this suppression. Table 1 illustrates the effects of the various ondansetron/5-HT conditions on sucrose intake. Intraperitoneal administration of 5-HT produced a significant dose-responsive reduction of intake compared to control in food-deprived rats ($r^2 = 0.989$). Administration of ondansetron alone did not significantly alter food intake compared to control ($P = 0.741$). However, when ondansetron was co-administered with 5-HT, the suppression of sucrose intake induced by 5-HT was significantly attenuated or completely reversed (Table 1).

Table 1
15% Sucrose intake in response to 5-HT and ondansetron administration

Drug condition	20 min intake (% of control)	P
Saline	100	–
Ondansetron 1.0 mg/kg	97.3 ± 6.8	NS
5-HT 0.25 mg/kg	80.7 ± 4.2	0.005 ^a
5-HT 0.5 mg/kg	69.8 ± 4.4	0.0001 ^a
5-HT 1.0 mg/kg	54.1 ± 4.3	<0.0001 ^a
5-HT 0.25 mg/kg + ondansetron 1.0 mg/kg	98.0 ± 3.5	0.015 ^b
5-HT 0.5 mg/kg + ondansetron 1.0 mg/kg	97.0 ± 19.0	0.004 ^b
5-HT 1.0 mg/kg + ondansetron 1.0 mg/kg	77.5 ± 6.9	0.024 ^b

Values are expressed as a percent of saline intake ± S.E. ($n=8$ rats). All drugs were administered in a volume of 1.0 ml/kg. NS, not significant.

^a P from saline.

^b P from corresponding 5-HT dose.

3.2. Experiment 2: 5-HT-induced suppression of food intake following blockade of CCK-1 receptors

Two-way repeated-measures ANOVA revealed a significant main effect on 30 min food intake for 5-HT treatment [$F(1, 93)=73.90; P<0.0001$]. There was no significant main effect of lorglumide [$F(1, 93)=1.24; P=0.269$] or an interaction between 5-HT and lorglumide on food intake [$F(1, 93)=1.59; P=0.211$]. As illustrated in Fig. 1, systemic administration of 5-HT reduced 30 min sucrose intake significantly compared to control (12.1 ± 1.3 ml versus 16.2 ± 0.9 ml; $P<0.001$). Lorglumide alone had no significant effect on food intake compared to saline injections (16.3 ± 1.2 ml versus 16.2 ± 0.9 ml; $P=0.99$). Blockade of CCK-1 receptors by lorglumide had no effect on 5-HT-induced suppression of intake ($P=0.47$).

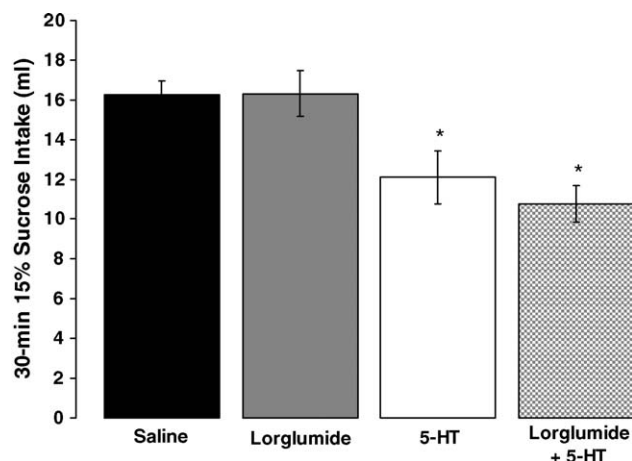


Fig. 1. Administration of 5-HT (1.0 mg/kg; IP) significantly reduced sucrose intake compared to saline administration. Blockade of CCK-1 receptors by lorglumide (1.0 mg/kg; IP) was unable to significantly alter 5-HT-induced suppression of 30 min sucrose intake. * $P<0.05$ from saline.

3.3. Experiment 3: CCK-1 and 5-HT3 receptor participation in suppression of food intake by simultaneous administration of CCK and 5-HT

3.3.1. Experiment 3a

One-way repeated measures ANOVA demonstrated an overall significant main drug treatment effect [$F(6, 167)=27.03; P<0.0001$] on 30 min food intake. The Bliss Independence Model [$F_{AB}=F_A+F_B(1-F_A)$] was applied to determine the nature of interaction between 5-HT and CCK when simultaneously administered. Thus, the suppression in food intake generated by administration of either 5-HT or CCK alone is reported as a fractional response from control intake [(control intake (ml) – treatment intake (ml))/control intake (ml)].

As illustrated in Fig. 2, independent administration of small, subthreshold doses of either CCK (0.05 μ g/kg) or 5-HT (0.1 mg/kg) had no significant effect on sucrose intake compared to control (13.5 ± 0.6 , 14.0 ± 0.7 and 15.0 ± 0.6 ml for 5-HT, CCK and saline, respectively; $P>0.05$). However, co-administration of subthreshold doses of 5-HT (0.1 mg/kg) and CCK (0.05 μ g/kg) produced a significant suppression in sucrose intake ($P<0.001$). The suppression of food intake fractional response generated by 5-HT (F_A) and CCK (F_B) was 0.1 and 0.066, respectively. Therefore, based on the Bliss Independence Model, the predicted additive suppression of food intake response (F_{AB}) generated by CCK and 5-HT co-administration is 0.159, with a mean 30 min intake of 12.6 ml sucrose. Intake following co-administration of subthreshold doses of CCK and 5-HT was 11.6 ± 0.5 ml (fractional response = 0.226), which was significantly below intake following administration of saline ($P<0.001$), 5-HT ($P=0.019$), CCK ($P=0.009$) or the Bliss predicted intake ($P<0.05$). These results indicate that CCK and 5-HT act in a synergistic manner to reduce food intake.

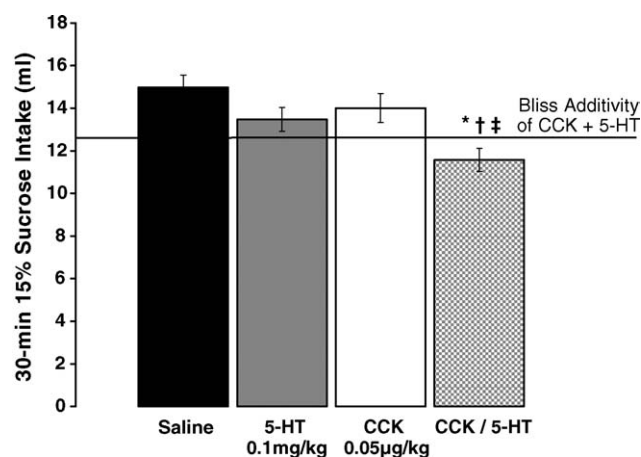


Fig. 2. When administered alone at subthreshold doses, neither 5-HT (0.1 mg/kg) or CCK (0.05 μ g/kg) significantly altered food intake compared to control. However, when co-administered, CCK and 5-HT synergistically reduced sucrose intake, as determined by the Bliss Independence Model. * $P<0.05$ from saline; † $P<0.05$ from CCK or 5-HT alone; ‡ $P<0.05$ from the Bliss additivity of CCK and 5-HT.

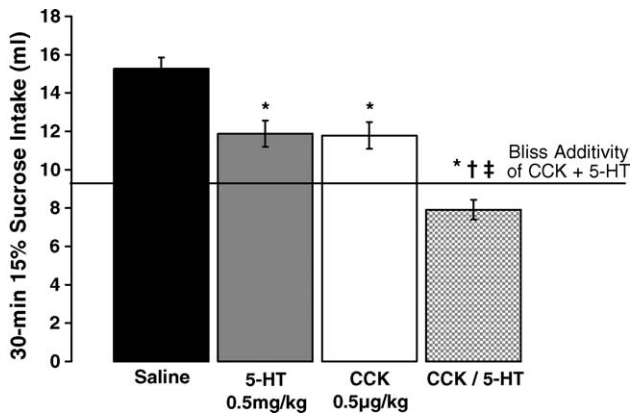


Fig. 3. Intraperitoneal administration of 5-HT (0.5 mg/kg) or CCK (0.5 µg/kg) alone significantly reduced sucrose intake compared to control. When co-administered, CCK and 5-HT synergistically reduced sucrose intake, as determined by the Bliss Independence Model. * $P < 0.05$ from saline; † $P < 0.05$ from CCK or 5-HT alone; ‡ $P < 0.05$ from the Bliss additivity of CCK and 5-HT.

r4 Fig. 3 shows sucrose intake following administration of 5-HT and CCK at doses that when given independently produce a significant suppression of intake. Sucrose intake was significantly reduced after administration of 5-HT (0.5 mg/kg; IP) compared to control (11.9 ± 0.7 ml versus 15.3 ± 0.6 ml for 5-HT and saline, respectively; $P < 0.0001$, $F_A = 0.22$). Similarly, CCK (0.5 µg/kg) alone significantly reduced intake compared to control (11.8 ± 0.7 ml; $P < 0.0001$, $F_B = 0.229$). Simultaneous administration of 5-HT (0.5 mg/kg) and CCK (0.5 µg/kg) caused a significant enhancement of the suppression (7.9 ± 0.5 ml; $P < 0.0001$, $F_{AB} = 0.484$). Based on the Bliss Independence Model, the predicted additive suppression of food intake response (F_{AB}) generated by CCK (0.5 µg/kg) and 5-HT (0.5 mg/kg) co-administration is 0.400, with a mean 30 min intake of 9.17 ml sucrose. Since intake following co-administration of CCK and 5-HT was significantly lower than the Bliss predicted intake (7.9 ml versus 9.17 ml, $P = 0.021$), as well as intake following administration of saline ($P < 0.0001$), 5-HT ($P < 0.0001$) or CCK ($P < 0.0001$) this again confirms that CCK and 5-HT act in a synergistic manner to reduce food intake.

3.3.2. Experiment 3b

Two-way repeated-measures ANOVA demonstrated a significant treatment effect for CCK/5-HT combination [$F(1, 93) = 31.96$, $P < 0.0001$] and for lorglumide/ondansetron combination [$F(1, 93) = 24.4$, $P < 0.0001$] on 30 min food intake. However, there was no significant main interaction effect between CCK/5-HT and lorglumide/ondansetron combination [$F(1, 93) = 2.41$, $P = 0.124$]. As shown in Fig. 4, co-administration of CCK (0.5 µg/kg) and 5-HT (0.5 mg/kg) produced a significant reduction in 30 min sucrose intake (9.5 ± 0.8 ml) compared to control (15.5 ± 0.6 ml; $P < 0.0001$) in food-deprived rats. Blockade of CCK-1 receptors by lorglumide (1.0 mg/kg) attenuated the enhanced sup-

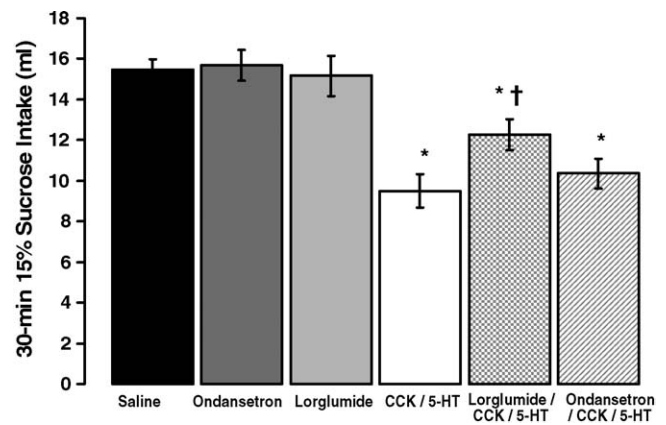


Fig. 4. Co-administration of 5-HT (0.5 mg/kg) and CCK (0.5 µg/kg) significantly reduced 30 min sucrose intake compared to control. Blockade of CCK-1 receptors by lorglumide (1.0 mg/kg; IP) significantly attenuated the synergistic enhanced suppression of food intake by CCK/5-HT co-administration. However, blockade of 5-HT₃ receptors by ondansetron (1.0 mg/kg; IP) was unable to significantly attenuate the enhanced suppression of food intake by CCK/5-HT co-administration. * $P < 0.05$ from saline; † $P < 0.05$ from CCK/5-HT.

pression of food intake by CCK and 5-HT co-administration (12.3 ± 0.8 ml versus 9.5 ± 0.8 ml for lorglumide/CCK/5-HT and CCK/5-HT, respectively; $P < 0.012$). Blockade of 5-HT₃ receptors by ondansetron (1.0 mg/kg) had no significant effect on the enhanced suppression of intake by CCK and 5-HT co-administration (10.4 ± 0.8 ml versus 9.5 ± 0.8 ml for ondansetron/CCK/5-HT and CCK/5-HT, respectively; $P = 0.979$). However, as Fig. 5 illustrates, simultaneous blockade of CCK-1 and 5-HT₃ receptors reversed the enhanced suppression of food intake by

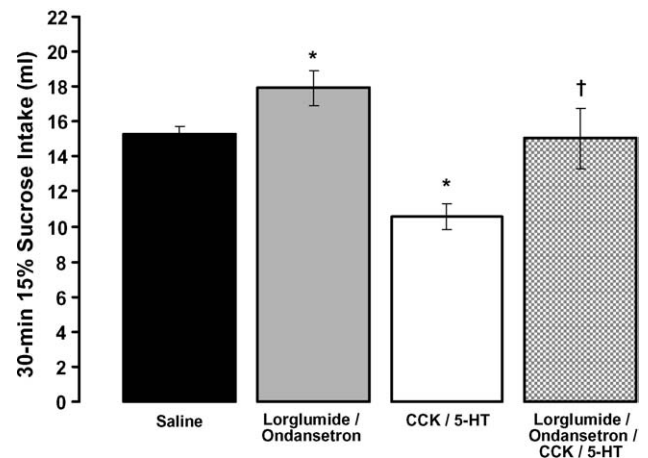


Fig. 5. Concomitant administration of lorglumide (1.0 mg/kg) and ondansetron (1.0 mg/kg; IP) produced a significant synergistic increase in 30 min sucrose intake compared to intakes after administration of saline or either antagonist alone. Co-administration of 5-HT (1.0 mg/kg) and CCK (1.0 µg/kg; IP) significantly reduced 30 min sucrose intake compared to control. Simultaneous blockade of CCK-1 and 5-HT₃ receptors by lorglumide (1.0 mg/kg) and ondansetron (1.0 mg/kg; IP) significantly reversed the synergistic enhanced suppression of food intake by CCK/5-HT co-administration. * $P < 0.05$ from saline; † $P < 0.05$ from CCK/5-HT-induced suppression of intake.

CCK and 5-HT (15.0 ± 1.7 ml versus 10.6 ± 0.7 ml for lorglumide/ondansetron/CCK/5-HT and CCK/5-HT combination, respectively; $P < 0.0001$). Likewise, intake following simultaneous blockade of CCK-1 and 5-HT₃ receptors (17.9 ± 1.0 ml) resulted in a significant increase in sucrose intake compared to intakes following administration of CCK/5-HT combination (9.5 ± 0.8 ml; $P < 0.0001$), lorglumide alone (15.7 ± 0.8 ml; $P < 0.05$), ondansetron alone (15.2 ± 1.0 ml; $P < 0.05$), as well as control (15.5 ± 0.6 ml; $P < 0.05$).

4. Discussion

The results of these studies reveal that systemic administration of 5-HT reduced sucrose intake in a dose dependent manner, and that this suppression is mediated via 5-HT₃, not CCK-1 receptors. Furthermore, we show that concomitant administration of CCK and 5-HT results in a synergistic interaction of the two drugs leading to an enhanced suppression of food intake. The effect of this interaction on food intake requires an interdependent cooperation between CCK-1 and 5-HT₃ receptors. Overall, these findings extend on several prior studies demonstrating an interaction between the cholecystokinergic and serotonergic systems in control of food intake [7,9,11,19,20,57]. These results support previous work by our laboratory [19,20,50,51] and others [1,6,7,11,18] reporting that 5-HT₃ receptors are involved in the negative feedback control of food intake under a number of feeding paradigms. Our results also show that 5-HT-induced suppression of intake is not altered in response to CCK-1 receptor blockade by lorglumide. The inability of CCK-1 receptors to mediate 5-HT-induced suppression of food intake has been previously reported [14] using devazepide, another selective CCK-1 receptor antagonist.

While the mechanisms by which 5-HT reduces food intake are not entirely known, systemic administration of 5-HT has been shown to inhibit sham intake [36,37] in the absence of a complete satiety sequence [53] through activation of 5-HT_{2a/2c} receptors [36]. However, to date there has been no direct study confirming the involvement of 5-HT₃ receptor in 5-HT-induced suppression of sham intake using a selective 5-HT₃ receptor antagonist. Therefore, it is still unknown whether 5-HT₃ receptors directly mediate 5-HT induced anorexia or whether their participation involves gastric/post-gastric feedback mechanisms. Nevertheless, given the current findings in conjunction with the facts that systemic 5-HT does not readily permeate the blood brain barrier [39] and that 5-HT₃ receptors are located on vagal afferent terminal fibers [8,43], it is highly likely that 5-HT₃ receptors directly mediate systemic 5-HT-induced suppression of intake. However, in our study ondansetron completely reversed inhibition of feeding by the two lowest doses of 5-HT (0.25, 0.5 mg/kg) and only partially attenuated the effects induced by the highest dose (1.0 mg/kg). This suggests that 5-HT₃ receptors are responsible for mediating peripheral effects of 5-HT when levels are nearer to the physiological range, but that higher

doses could potentially permeate the CNS at circumventricular regions or activate other 5-HT receptor populations, such as 5-HT₂ or 5-HT₁ receptors. Therefore, based on these results, we cannot completely ascribe all of systemic 5-HT's anorectic effect to 5-HT₃ receptor activity, which is also in line with previous reports [9].

Our data also show that concomitant administration of 5-HT and CCK enhanced suppression of food intake. This is in agreement with several studies demonstrating an interaction between 5-HT and CCK in control of feeding behavior in response to lipid-induced satiation [7] amino acid imbalanced diets [1] or when the two drugs are centrally administered [21]. However, early work designed to investigate the relationship between 5-HT and CCK in control of food intake had minimized or discounted the role of peripheral serotonergic involvement in the CCK/5-HT interaction [9,10,40]. The possible contribution of 5-HT₃ receptors in mediating this interaction has also not been addressed or was originally presumed not to be involved [6,9]. The present studies provide convincing evidence that peripheral 5-HT and CCK interact to suppress food intake and that 5-HT₃ and CCK-A receptors together mediate this effect. Accumulating evidence strengthens this notion and indeed several studies demonstrate that 5-HT₃ receptors mediate a number of satiation signals presumed to increase endogenous levels of 5-HT and/or CCK [18,40,50,51]. Current findings indicate that when CCK and 5-HT were administered together, the suppression of food intake was synergistically enhanced. Thus, a CCK dose ineffective in eliciting a significant suppression of intake when administered alone became effective when combined with a dose of 5-HT that was also subthreshold for suppressing intake of sucrose. Hence, anorectic signals elicited by exogenous administration of CCK and 5-HT may be integrated and amplified within the periphery to provoke responses greater in magnitude than application of either stimulus separately. Furthermore, a CCK dose that alone was effective in producing a significant suppression of intake yielded an even greater, amplified anorectic response when administered in combination with a suprathreshold dose of 5-HT. This synergistic effect was also confirmed by the Bliss Independence Model [2,12], which showed that the reduction in intake by the co-administration of subthreshold or suprathreshold doses of CCK and 5-HT were significantly greater than the predicted additive suppression. This enhanced suppression of intake is most likely mediated through cooperative activation of CCK-1 and 5-HT₃ receptors. Indeed, when both selective antagonists were concomitantly administered, this resulted in a complete reversal of the synergistic suppression by CCK and 5-HT co-administration. This finding supports other recent work demonstrating a cooperation between CCK-1 and 5-HT₃ receptors in mediating the satiating effects produced by intrainestinal nutrients [7], amino acid deficiencies [1], as well as CCK-8-induced satiation [20] and further strengthens the overall notion that systemic cholecystokinergic and serotonergic systems interact in control of food intake.

Systemic administration of ondansetron alone did not significantly alter food intake, suggesting that the satiety response generated by sucrose ingestion alone is not mediated through 5-HT₃ receptors. This is consistent with our previous studies [19,20] as well as those of van der Hoek and Cooper [59] who also showed that systemic ondansetron treatment did not alter food intake in overnight fasted rats. It is noteworthy that contrary to van der Hoek and Cooper, we have previously been unable to find any effect on food intake by ondansetron in nondeprived rats [20].

Although 5-HT₃ receptors have been implicated in emetic reflexes, we did not observe any malaise-like behavior following administration of 5-HT or ondansetron at any dose. This observation is supported by Pollock and Rowland [41] who have previously shown that systemic administration of 5-HT at a dose of 2.0 mg/kg or less did not produce a conditioned taste aversion when paired repeatedly with sucrose ingestion. Therefore, our 5-HT doses tested (1.0 mg/kg or less) fall well below the level of reported taste aversion with sucrose ingestion. However, we cannot rule out the possibility that there may in fact be some sub-threshold emetic response to systemic 5-HT activating 5-HT₃ receptors.

While much of the existing 5-HT₃ receptor evidence points to a peripheral site of action, the effects observed in the current studies cannot be attributed entirely to the periphery. Ondansetron has been shown to minimally penetrate the blood brain barrier. According to Simpson et al. [55] up to 15% of systemic ondansetron can be detected in the cerebral spinal fluid [39,55]. Given that 5-HT₃ receptors are present in the nucleus of the solitary tract and area postrema, two regions known to be involved in the negative feedback control of food intake, as well as having circumventricular properties, it is possible that the feeding effects observed in the present studies may also be due to ondansetron's ability to block hindbrain 5-HT₃ receptors. Additionally, there is convincing evidence demonstrating that systemic administration of CCK reduces food intake by activating CCK-1 receptors that are predominantly located in the periphery [4,45]. Therefore, we can assert that the behavioral results seen in our studies in response to CCK and lorglumide administration, originate from participation of CCK-1 receptors within the periphery. However, detailed examination of peripheral and hindbrain 5-HT₃ receptor participation in systemic CCK's satiating effects are still needed.

The vagal afferent level is another plausible site of systemic CCK and 5-HT interaction. Vagal afferents exhibit excitatory responses to both CCK-1 [3,22,30,32,54] and 5-HT₃ [3,22,30] receptor activation. Furthermore, 38% of nodose neurons respond to CCK [54] and up to 80% to 5-HT [58]. This suggests that some neurons of the nodose ganglion must respond to both CCK and 5-HT. In fact, Li et al. have recently shown that more than 50% of the nodose ganglion neurons responded to both systemic CCK-8 and 5-HT [30]. Thus, it is possible that the enhanced suppression of food intake by concomitant activation of CCK-1 and 5-HT₃ receptors is a

result of a neuronal integration of the excitatory signal at the level of the nodose neuron.

Control of food intake is governed by a number of integrated within-meal and homeostatic signals. A large majority of within-meal satiety occurs in response to a combination of negative feedback arising from volumetric distension of the stomach, as well as nutrients entering into the duodenum [42,47]. Both 5-HT₃ and CCK-1 receptors have been shown to independently mediate gastric- [33–35,43,44] and intestinal nutrient- [5,29,50,51,60] induced suppression of food intake and feeding related physiological functions. Thus, concomitant reciprocal mediation of gastric and intestinal negative feedback by these two receptors would invoke an overall enhancement in meal terminating signals. We have previously demonstrated that 5-HT₃ receptors mediate CCK-induced satiation through an indirect mechanism involving gastric/post-gastric feedback [19]. It is well known that one function of CCK is to inhibit gastric emptying resulting in a subsequent increase in gastric distension due to retention of stomach contents. Gastric distension itself has been shown to intensify the satiating effect of CCK [25], as well as synergistically stimulate gastric vagal afferent fibers [52]. In addition, gastric distension induces 5-HT release resulting in neuronal activation in the nucleus of the solitary tract (NTS), area postrema (AP), paraventricular nucleus (PVN), and hypothalamic regions which have been shown to be involved in control of food intake [33]. This effect was reported to be mediated by systemic 5-HT₃ receptors [33]. Consequently, one mechanism by which 5-HT₃ receptors participate in CCK-induced satiation may involve gastric distension-induced release of 5-HT.

In conclusion, the present findings indicate that systemic 5-HT reduces food intake by activating 5-HT₃, not CCK-1 receptors. In addition, we have demonstrated that peripherally co-administered CCK and 5-HT interact to synergistically suppress food intake. Finally, we have provided evidence that the enhanced suppression of intake following concomitant administration of CCK and 5-HT is due to simultaneous CCK-1 and 5-HT₃ receptor activation.

Acknowledgements

The authors wish to thank Bart C. De Jonghe, Rachael L. Moore, Samit M. Shah, and Jonathan G. Stine for their help with these studies and preparation of this manuscript.

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