Intraduodenal infusion of a combination of tastants decreases food intake in humans

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ABSTRACT

Background: Taste receptors are expressed not only in taste buds but also in the gastrointestinal tract. It has been hypothesized that these receptors may play a role in satiety and food intake. Objective: This study investigated the effect of intraduodenal tastant infusions (bitter, sweet, and umami) on food intake, hunger and fullness, gastrointestinal symptoms, and gastrointestinal peptide release. Design: Fifteen healthy volunteers (6 male; mean ± SEM age: 23.9 ± 2.0 y; mean ± SEM body mass index (in kg/m²): 22.4 ± 0.3) received 5 treatments in a double-blind, randomized, placebo-controlled crossover design. Test days started with the insertion of a nasoduodenal catheter followed by a standardized liquid breakfast. Participants received an intraduodenal infusion 150 min after breakfast, containing quinine (bitter), rebaudioside A (sweet), monosodium glutamate (umami), a combination of the 3 tastants, or placebo (tap water) over a period of 60 min. Food intake was measured during an ad libitum meal, and visual analog scales were used to monitor gastrointestinal complaints and hunger and fullness scores. Blood samples were drawn at regular intervals for cholecystokinin, glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) analysis. Results: Infusion of the combination of tastants substantially decreased food intake (422 ± 97 compared with 486 ± 104 kcal for placebo, P < 0.05), whereas both a combination of tastants and umami decreased hunger scores compared with placebo. No change in cholecystokinin, GLP-1, or PYY concentrations was observed during the infusions. Intraduodenal infusions of the tastants did not result in gastrointestinal symptoms. Conclusions: Intraduodenal infusion of umami and a combination of tastants inhibits feelings of hunger, but only the latter also reduces food intake. However, these alterations were not accompanied by changes in the plasma concentrations of the gut-derived peptides cholecystokinin, GLP-1, or PYY. This trial was registered at clinicaltrials.gov as NCT01956838.

Keywords: tastants, intraduodenal infusion, food intake, gastrointestinal peptides

INTRODUCTION

Tastants are sensed by taste buds, which constitute taste cells on the tongue. Humans are able to detect 5 basic tastes: bitter, sweet, umami, sour, and salty. Sensing sour and salty is mediated by ion channels, whereas sweet and umami are sensed by the taste receptor type 1 family, and bitter triggers the taste receptor type 2 family (1). Activation of these receptors on the taste cells of the tongue results in release of gastrointestinal peptides such as cholecystokinin, glucagon-like peptide 1 (GLP-1),6 and peptide YY (PYY), which can modulate taste responses (2, 3). It has been shown previously that noncaloric bitter, umami, and sweet tastants induce cholecystokinin release in STC-1 cells (4, 5). Several investigators have reported on the expression of taste receptor types 1 and 2 on enteroendocrine cells in several parts of the gastrointestinal tract (6, 7). Both receptor families were expressed in human stomach, small intestine, and colon (8, 9).

These findings point to a potential role for noncaloric tastants in the release of gastrointestinal peptides and regulation of food intake in humans. At present, data on effects of tastants on gastrointestinal peptide release in the human gastrointestinal tract are limited. Intragastric administration of the bitter tastant denatonium benzoate significantly increased satiation in healthy volunteers, whereas umami added to a meal resulted in a significant increase in plasma GLP-1 concentrations compared with placebo (10, 11). In these studies, tastants were administered orally or intragastrically, whereas the latter route of administration has the advantage of bypassing orosensory stimulation. However, after both oral and intragastric administration, variations in gastric emptying rate can cause differences in gastrointestinal peptide release and satiety. Intraduodenal infusion has the advantage of studying the direct interactions between tastants and the gastrointestinal tract, and therefore we evaluated the effect of intraduodenal tastant infusion on ad libitum food intake, satiety, gastrointestinal symptoms, and gastrointestinal peptide release in vivo in humans. We hypothesized that intraduodenal infusion of the bitter, sweet, and umami tastants or their combination would result in a decrease in food intake (422 ± 97 compared with 486 ± 104 kcal for placebo, P < 0.05), whereas both a combination of tastants and umami decreased hunger scores compared with placebo. No change in cholecystokinin, GLP-1, or PYY concentrations was observed during the infusions. Intraduodenal infusions of the tastants did not result in gastrointestinal symptoms.
in food intake and an increase in the release of cholecystokinin, GLP-1, and PYY compared with placebo.

METHODS

This study was approved by the Medical Ethics Committee of the Maastricht University Medical Center+, Maastricht, Netherlands, and performed in full accordance with the Declaration of Helsinki. The study was registered at www.clinicaltrials.gov as NCT01956838.

Study design

In this double-blind, randomized, placebo-controlled crossover study, tastants (bitter, sweet, umami, and a combination of these) were administered into the duodenum and compared with placebo (tap water). All volunteers participated in 5 test days with 1 wk in between test days to prevent carryover effects.

Tastant infusions

Three different tastants were directly infused into the duodenum. To reduce the chance of side effects, we decided to infuse all tastants at 75% of the acceptable daily intake. For the bitter agent, 75 mg quinine (Arnold Suhr) was dissolved in 120 mL tap water (3 mOsmol/L). For the sweet tastant, 540 mg rebaisdioxide A (Reb A; Steviya Natuurlijk) was dissolved in 120 mL tap water (1 mOsmol/L). For the umami tastant, 2 g monosodium glutamate (MSG; Ajinomoto) was dissolved in 120 mL tap water (153 mOsmol/L). We combined all 3 tastants (75 mg quinine, 540 mg Reb A, and 2 g MSG) in the combination treatment (dissolved in 120 mL tap water, 168 mOsmol/L). The placebo treatment consisted of 120 mL tap water. The infusion was mixed with a magnetic stirrer until the tastants were fully dissolved.

Subjects

Volunteers were eligible to participate if they were healthy, aged between 18 and 55 y, and had a BMI (in kg/m²) between 18 and 25. Volunteers were recruited by local advertisement. Smoking, consumption of >100 g alcohol/wk, history of any relevant disorder or surgery (assessed by a physician), gastrointestinal symptoms, and medication use (apart from oral contraceptives) were exclusion criteria. All participants needed to be weight stable for at least 2 mo before participation (<5% change in body weight) and to be unrestrained eaters, as assessed by the Dutch eating behavior questionnaire (12). Initial information was provided verbally or via e-mail, and after providing detailed written study information, a minimum time period of 7 d was given before inclusion into the study. After obtaining written informed consent, subjects participated in a taste perception test. Participants were blinded and tasted all study substances (quinine as the bitter agent, MSG for umami, and Reb A for sweet). Subjects were included in the intraduodenal infusion experiments when they were able to taste and identify all 3 tastants. According to our power calculation, 15 subjects were needed to complete the study. This number was based on the difference in food intake observed in previous work (13). Sixteen subjects met the inclusion criteria. Due to discomfort induced by the intraduodenal catheter, one subject did not complete all experiments and dropped out of the study.

Fifteen healthy subjects (6 male; mean ± SEM age: 23.9 ± 2.0 y; mean ± SEM BMI: 22.4 ± 0.3) completed the study.

Protocol

On each test day, subjects arrived at 0800 h, after an 8-h overnight fast, at the Maastricht University Medical Center+. Subjects were instructed to consume the same habitual meal on the evening before each test day. Test days started with the insertion of a commercially available 145-cm nasoduodenal catheter (Fiocare Bengmark; Nutricia). After local anesthesia of the nasal mucosa (xylocaine 10% spray; AstraZeneca), the catheter was introduced transnasally into the stomach. Under intermittent fluoroscopic control, the catheter was positioned with the tube tip located in the descending part of the duodenum. After correct positioning of the nasoduodenal catheter, an intravenous cannula was placed in a forearm vein for collection of blood samples. Subsequently, a fasted sample and visual analogue scale (VAS) for hunger, satiety, and gastrointestinal symptoms were obtained, followed by consumption of a standardized liquid breakfast meal (250 mL Goedemorgen drinkontbijt (Friesland Campina); 162 kcal, 7 g protein, 29 g carbohydrates, and 2 g fat). This was considered t = 0 min. Then, 150 min after breakfast intake, an infusion pump was connected to the feeding catheter to infuse a solution containing quinine (bitter), Reb A (sweet), MSG (umami), combination, or placebo (t = 150 min) on different test days. Substrate was infused at a rate of 2 mL/min for 60 min, and at the end of the infusion, the feeding catheter was removed. Fifteen minutes after ending the infusion, volunteers received a standardized ad libitum lunch meal, which was offered in excess, and were instructed to eat until comfortably full (lasagna meal (Plus supermarkt); energy density per 100 g: 160 kcal, 7 g protein, 11.0 g carbohydrates, and 9.4 g fat) (t = 225 min). After ingestion of the lunch meal, the test day was finished, and subjects were allowed to return home.

Gastrointestinal peptides

Venous blood samples were drawn at regular intervals (baseline and 150, 165, 180, 195, 210, and 225 min). For GLP-1 (7–36), PYY, and cholecystokinin measurements, blood was collected in ice-chilled EDTA aprotinin-coated tubes (Becton & Dickinson), and 10 μL dipeptidyl peptide-4 inhibitor (DPP4-010; Merck Millipore) per 1 mL whole blood was immediately added after blood collection to prevent proteolytic cleavage. Tubes were centrifuged at a rate of 1157 × g at 4°C for 15 min, and plasma was transferred into aliquots and stored at −80°C.

Active GLP-1 (7–36) was determined with a Glucagon Like Peptide-1 (Active) ELISA kit with a range of 2–100 pmol/L, an interassay CV of 11%, and an intra-assay CV of 6% (EGLP-35K; Merck Millipore). Total PYY (includes both peptide YY1–36 and peptide YY3–36) was measured with a Human PYY (Total) ELISA kit with an interassay CV of 6% and an intra-assay CV of 3% (EZHPYYT66K; Merck Millipore). Plasma cholecystokinin 8 (cholecystokinin 26–33) concentrations were measured with an optimized and validated commercial human radioimmunoassay kit (EURIA CCK, RB302; Euro-Diagnostica) (14). This improved assay system has been optimized to reach a high sensitivity of 0.05 pmol/L and has no cross-reactivity to gastrin 17 or sulfated gastrin. The intra-assay CV was 8.9% at a
and satiety are given in Satiety and desire to eat and increased fullness and satiety scores in all interventions. Ingestion of the breakfast meal decreased hunger and satiety did not differ among the 5 interventions. Ingestion of the breakfast meal decreased hunger and satiety did not differ among the 5 interventions.

Statistical analyses

Statistical analyses were performed with the SAS statistical software package (SAS version 9; SAS Institute). Data were visually checked on normality and on constant variance of residuals by plots of residuals vs. corresponding predicted values. If data were not normally distributed, log transformation was applied for further analysis of the data, as was the case for cholecystokinin, GLP-1 (7–36), and PYY.

Regarding food intake, statistical analysis was performed on the amount of food eaten in kilocalories. Cholecystokinin, GLP-1 (7–36), and PYY are displayed from the start of the substrate infusion (t = 150 min) until the last blood sample collected before the start of the ad libitum meal (t = 225 min). The effects of each intervention on gut hormones were determined by analyzing the peptide concentrations at the onset of tastant infusion until ingestion of the ad libitum meal. All data were corrected for the values obtained at the onset of infusion.

All variables were compared with a mixed ANOVA model that included the fixed factor treatment (placebo, bitter, umami, sweet, and combination). For the VAS, gastrointestinal symptoms and plasma parameters, time, and the interaction between treatment and time were added to the model. Because of the crossover design, intervention effects within subjects were compared by including the random factor subject. If an intervention effect occurred, a post hoc Dunnett test was used to analyze differences in ad libitum meal intake. A post hoc Tukey-Kramer test was used to analyze differences in VAS scores, gastrointestinal symptoms, and gut hormones (cholecystokinin, GLP-1, and PYY). Data are presented as means ± SEMs (unless specified otherwise) and considered significant at P < 0.05.

RESULTS

Meal intake

Ad libitum food intake was decreased after intraduodenal infusion of the combination of tastants compared with placebo (422 ± 97 kcal compared with 486 ± 104 kcal, respectively; P < 0.05) (Figure 1). No differences in food intake were observed after infusion of any of the separate tastants compared with placebo (bitter: 442 ± 91 kcal, umami: 491 ± 113 kcal, and sweet: 462 ± 101 kcal).

Satiety

Mean VAS scores and AUC for hunger, fullness, desire to eat, and satiety are given in Figure 2. Fasting scores for hunger, fullness, desire to eat, and satiety did not differ among the 5 interventions. Ingestion of the breakfast meal decreased hunger and desire to eat and increased fullness and satiety scores in all treatments to the same extent (data not shown). Decreases in hunger and desire to eat scores were observed after intraduodenal infusion of umami (hunger: umami compared with placebo, P < 0.05; desire to eat: umami compared with placebo, P < 0.05; umami compared with bitter, P < 0.05) and after infusion of the combination of tastants (hunger: combination compared with placebo, P < 0.05; desire to eat: combination compared with placebo, P < 0.01; combination compared with bitter, P < 0.05) but not after the other infusions compared with placebo (Figure 2A and 2C).

Gastrointestinal symptoms

Mean VAS scores and AUCs for nausea, stomach pain, and bloating are presented in Figure 3. No differences were observed in nausea, stomach pain, or bloating at the start of the infusion. Intraduodenal infusion of the tastants or placebo did not result in changes in nausea, stomach pain, or bloating over time, both in VAS scores and in AUCs.

Gastrointestinal peptides

Fasted plasma cholecystokinin, GLP-1 (7–36), or PYY concentrations did not differ between interventions, whereas none of the tastant infusions resulted in different plasma cholecystokinin, GLP-1 (7–36), or PYY concentrations compared with placebo (Figure 4).

DISCUSSION

This study clearly shows that intraduodenal infusion of umami and a combination of tastants affects hunger feelings and that only the combination also decreases ad libitum meal intake compared with placebo in vivo in humans. Infusion of the other single tastants did not modulate feelings of satiety or of food intake. Plasma concentrations of gastrointestinal peptides that are generally considered to play a role in the control of food intake.
intake, cholecystokinin, GLP-1, or PYY, were not affected by intestinal tastant administration.

Several studies reported previously on the expression of taste receptor type 1, taste receptor type 2, and other taste signaling proteins in several parts of the small and large intestine (7, 8, 16). Activation of these taste receptors, expressed on enteroendocrine cells, may trigger release of several gastrointestinal peptides, including cholecystokinin, GLP-1, and PYY. Several in vitro and ex vivo studies have shown that tastants are able to induce release of cholecystokinin and GLP-1 (4, 8, 17). Up to now, it remained unclear whether activation of taste receptors via infusion of tastants may also lead to release of gastrointestinal peptides and whether these enter the circulation and exert endocrine effects. The current study investigated the effects of presenting different tastants to the duodenum in vivo on the release of gastrointestinal peptides and on food intake. In contrast to the previously established in vitro and ex vivo data, we did not observe differences in the release of cholecystokinin, GLP-1, and PYY after tastants infusion in vivo in humans, despite the observation that satiety and food intake were modulated, at least by a combination of different tastants. However, differences in PYY concentrations may have reached statistical significance in a larger sample size.

In vivo human data on the effect of oral ingestion of tastants on gastrointestinal peptide release are limited. Hosaka et al. (11) added MSG (umami) to a liquid meal, and this resulted in a postprandial increase in GLP-1 plasma concentrations. Furthermore, these authors found a reduction in plasma glucose concentrations over the first 60 min after meal ingestion. In the

**FIGURE 2**  Mean ± SEM VAS for hunger (A), fullness (B), desire to eat (C), and satiety (D) during intraduodenal infusion of placebo and bitter, umami, sweet, and combination tastants. Intraduodenal infusion was started at t = 150 (150 min after breakfast consumption) and was continued for 60 min. An ad libitum lunch was offered at t = 225 min. Significantly lower hunger scores were observed after intraduodenal infusion of umami (#) and of the combination (*) than after infusion of placebo at $P < 0.05$. Significantly lower desire to eat scores were observed after intraduodenal infusion of umami (#) and of the combination (*) than after infusion of placebo at $P < 0.05$ and of the combination ($*P < 0.01$) than after infusion of placebo and bitter ($*P < 0.05$ for umami and $*P < 0.05$ for the combination). Significantly higher satiety scores were observed after intraduodenal infusion of umami (#) and of the combination (*) than after infusion of placebo at $P < 0.01$. These results were analyzed with a mixed ANOVA model with a post hoc Tukey-Kramer correction. VAS, visual analog scale.
current study, the same amount of MSG has been used as by Hosaka et al., but no differences in either GLP-1 or glucose concentrations were found. This discrepancy may in part be explained by the coingestion of the 520-kcal liquid meal in the Hosaka et al. study, which most likely would have induced an increase in the release of cholecystokinin, GLP-1, and/or PYY, thereby showing additional effects of MSG on gastrointestinal peptide release.

Our data show that umami (MSG) and the combination of tastants resulted in a significant decrease in hunger feelings compared with control, whereas we did not observe any changes in plasma concentrations of gastrointestinal peptides. This does not exclude a role for gastrointestinal peptides as paracrine or neuroendocrine mediators of hunger and fullness and a decrease in food intake. It has been shown that intragastric administration of a bitter tastant induces expression of c-fos in the nucleus of the solitary tract, thus supporting the hypothesis that tastants have the potential to trigger neuronal activation (18). Another interesting finding is that the reduction in hunger feelings as observed during umami (MSG) infusion did not result in a decrease in food intake. A similar discrepancy was seen in previous studies in which MSG was added to a meal (19, 20). It was reported that MSG led to a more rapid recovery of a motivation to eat. Such a fast recovery of hunger may have resulted in an absence of food intake reduction in the current study (20).

It is known that oral ingestion or intraduodenal infusion of the artificial sweetener sucralose does not alter appetite or gastrointestinal peptide release in vivo in humans (21–23). For this reason, and because the gastrointestinal peptide-releasing capacity of the natural sweetener Reb A was demonstrated in an ex vivo model (17), we chose to infuse Reb A in the current study instead of another, previously used, artificial sweetener. Our findings described in the current article demonstrate that intraduodenal infusion of Reb A, consistent with previous reports about artificial sweeteners, does not induce gastrointestinal peptide secretion.

It has repeatedly been shown that infusion of tastants in various parts of the small intestine results in a reduction in food intake (24–26). All of these studies have been performed with different macronutrient compositions and caloric densities. In the present study, we clearly show that intraduodenal infusion of a combination of noncaloric tastants also results in a decrease in ad libitum food intake. This finding supports the hypothesis that satiation may occur through direct signaling routes, without coingestion of nutrients or calories (27). Interestingly, none of the single tastants resulted in a significant decrease in food intake. A possible explanation for this phenomenon may be that the separate ingredients activate only a single receptor, whereas activation of multiple receptors, as is the case in the combination treatment, is needed to affect food intake. Therefore, it can be hypothesized that triggering multiple receptors could lead to an additive effect, resulting in a more pronounced effect on food intake.

We found a significant net reduction in food intake of 64 kcal after the intervention with the combination of tastants. This reduction in food intake appears to be rather small, but it is worth mentioning that this reduction was obtained after a single intraduodenal infusion of a combination of noncaloric tastants. If such daily reduction in calorie intake could be maintained by long-term administration of this combination, possibly with targeted delivery encapsulation systems, this would eventually lead to a decrease in food intake and, consequently, body weight reduction. It should be noted that in the current study, tastants were infused without the presence of a food matrix. It is tempting to speculate that in the presence of a calorie-containing meal, effects of tastants on food intake would be larger than those observed in the current study. In light of the previous findings that intraduodenal infusion of macronutrients induces strong effects on food intake behavior, it will also be important to assess the effects of ideal exposure to tastants on food intake.

The concentrations of tastants used in our study were based on the acceptable daily intake and previous application in other studies (11). We opted for infusion of no more than 75% of the acceptable daily intake for safety reasons. A limitation of the current study is that no dose-response study for any of the tastants has been performed, and therefore it remains uncertain whether the most optimal doses for each tastant separately and for the combination were used. Tastant selection was based on previous studies, in which it was shown that all ligands used in this study were able to activate the taste receptor [MSG (28), quinine (29, 30), and Reb A (31)]. However, it remains to be established whether the tastants actually activate the taste receptors in the human gastrointestinal tract at the concentrations used in this...
study. This is specifically true for MSG, which not only is a tastant but also has some caloric value, so effects of MSG may, at least in part, consist of metabolic effects of glutamate. However, infusion of 2 g MSG represents a caloric value of 5.8 kcal (24.2 kJ) only. Therefore, it seems unlikely that alterations in satiety/fullness and the relatively large change in food intake after the combination treatment (65 kcal) could be attributed to MSG’s caloric value.

Another limitation of this study is the fact that no taste receptor antagonists were used. Because this study was designed as a proof-of-concept study, future studies should include taste receptor antagonists to investigate their effect on taste receptor activation by tastants.

In conclusion, we have shown that intraduodenal infusion of the umami tastant and the combination of the tastants bitter, sweet, and umami significantly inhibits feelings of hunger and that only the combination also reduces food intake but does not affect release of the gastrointestinal peptides cholecystokinin, GLP-1, or PYY or gastrointestinal symptoms. Further studies are needed to elucidate the role of tastants and taste receptor activation in the regulation of food intake and its potential to provide a nutritional tool to control energy intake.

The authors’ responsibilities were as follows—FJT, HFJH, and AAMM: designed the research; MvA, FJT, and JP: conducted the research; MvA and DR: analyzed data and performed the statistical analyses; DR: performed the hormone analyses; MvA, FJT, HFJH, and AAMM: contributed to interpretation of the results; MvA, FJT, and AAMM: wrote the manuscript and had primary responsibility for the final content of the manuscript. TI Food and Nutrition had input in the study design, whereas study conduct, data collection and analysis, and manuscript writing were the sole responsibility of the academic partners. None of the authors reported a conflict of interest.

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