Sweetness and bitterness taste of meals per se does not mediate gastric emptying in humans

Tanya J. Little, Nili Gupta, R. Maynard Case, David G. Thompson, and John T. McLaughlin

1Section of Gastrointestinal Sciences, Manchester Academic Health Science Centre, University of Manchester, Salford Royal National Health Service Foundation Trust, Salford; and 2University of Manchester, Faculty of Life Sciences, Manchester, United Kingdom

Submitted 10 February 2009; accepted in final form 11 June 2009

Little TJ, Gupta N, Case RM, Thompson DG, McLaughlin JT. Sweetness and bitterness taste of meals per se does not mediate gastric emptying in humans. Am J Physiol Regul Integr Comp Physiol 297: R632–R639, 2009. First published June 17, 2009; doi:10.1152/ajpregu.00090.2009.—In cell line and animal models, sweet and bitter tastants induce secretion of signaling peptides (e.g., glucagon-like peptide-1 and cholecystokinin) and slow gastric emptying (GE). Whether human GE and appetite responses are regulated by the sweetness or bitterness per se of ingested food is, however, unknown. We aimed to determine whether intragastric infusion of “equisweet” (Study A) or “equibitter” (Study B) solutions slow GE to the same extent, and whether a glucose solution made sweeter by the addition of saccharin will slow GE more potently than glucose alone. Healthy nonobese subjects were studied in a single-blind, randomized fashion. Subjects received 500-nl intragastric infusions of predetermined equisweet solutions of glucose (560 mosmol/kgH2O), fructose (290 mosmol/kgH2O), aspartame (200 mg), and saccharin (50 mg); twice as sweet glucose + saccharin, water (volumetric control) (Study A); or equibitter solutions of quinine (0.198 mM), naringin (1 mM), or water (Study B). GE was evaluated using a [13C]acetate breath test, and hunger and fullness were scored using visual analog scales. In Study A, equisweet solutions did not empty similarly. Fructose, aspartame, and saccharin did not slow GE compared with water, but glucose did (P < 0.05). There was no additional effect of the sweeter glucose + saccharin solution (P > 0.05, compared with glucose alone). In Study B, neither bitter tastant slowed GE compared with water. None of the solutions modulated perceptions of hunger or fullness. We conclude that, in humans, the presence of sweetness and bitterness taste per se in ingested solutions does not appear to signal to influence GE or appetite perceptions.

sweet; gastrointestinal tract; bitter; sweet

NUTRIENT-INDUCED GUT-TO-BRAIN signaling plays a major role in the control of mammalian digestive function, appetite, and energy intake (38). These effects are mediated by a number of interrelated factors, including the modulation of gastric emptying (GE) (46) and gastrointestinal transit (1, 4, 10, 15, 16), and the release from enterodendocrine cells of a number of signaling peptides, including glucagon-like peptide-1 (GLP-1) (17, 23, 24), peptide YY (PYY) (2, 32), and cholecystokinin (CCK) (35). These peptides signal to the central nervous system (CNS), particularly the brain stem and hypothalamus, via the vagus nerve and the bloodstream, and lead to the modulation of GE. However, although these target organ effects are well recognized, the precise sensing and signaling mechanism(s) by which the gut detects the chemical composition of ingested foods, and thereby induces signaling to the CNS to modulate gastrointestinal function and energy intake, are poorly defined.

The GE of hexose sugars has traditionally been regarded to be dependent on the osmolality of the ingested test meal (9, 19). However, differences between the GE of equiosmolar solutions of glucose and fructose have recently been reported (9, 13, 31), suggesting that sugar(hexose)-specific effects also occur. Thus the role of osmolality may be overstated and be a proxy for sugar-specific effects (37) and their potency at the relevant sensor. It is also known that sodium chloride slows GE less potently than an equiosmolar solution of glucose (18). Therefore, it seems far more likely that sugars are also being specifically sensed by the upper gut, independently of their luminal concentration.

The recent demonstration of “taste receptor” expression in the gastrointestinal tract raises the possibility that the upper gut may be able to sense, and then induce, gut-to-brain signaling responses to the “taste” of ingested food. Sweet taste receptors (T1R2 and T1R3), bitter taste receptors (T2Rs), and α-gustducin, a G protein involved in taste signal transduction, have recently been reported to be expressed in enterodendocrine cells of the small intestine and colon of humans and animals (8, 41, 42, 48, 51, 52). Coexpression of α-gustducin with T1R2 and T1R3 has been demonstrated in the mouse duodenum (29), and α-gustducin is expressed in enterodendocrine I cells, which secrete CCK (47), and in L cells of the distal small intestine, which secrete GLP-1 and PYY (42).

Evidence that the mammalian gastrointestinal tract may be able to sense sweetness comes from studies in cell lines, and animal models. In a human L cell line (NCI-H716), glucose and sucralose (a noncaloric sweetener) induce GLP-1 secretion, an effect blocked by the sweet taste receptor antagonist lactisole, and, in vivo, the stimulation of GLP-1 secretion in response to intragastric infusion was abolished in α-gustducin null mice (21). These observations suggest that sweetness per se, as well as the molecular structure or osmolality of the ingested sugar, could initiate gut-to-brain signaling responses by releasing gut signaling peptides, activating vagal affenter neurons, and inducing a CNS-mediated feedback inhibition of GE and appetite. Therefore, if sweetness per se were sensed in the gastrointestinal tract, then infusion of “equisweet” solutions of nutrient, and artificial, sweeteners (administered directly to the upper gut to bypass lingual taste receptors) may be expected to exert similar effects on GE and appetite. A recent study in humans has demonstrated that a sucralose solution, matched for sweetness to sucrose, has no effect on GE or GLP-1 secretion (27); it is, however, unknown whether solu-
tions of hexose sugars matched for sweetness will exert equivalent effects on GE.

There is also evidence from animal studies that the gastrointestinal tract may be able to sense the bitterness of ingested food. For example, the bitter tantant denatonium benzoate has been reported to induce CCK secretion from an enteroendocrine cell line (STC-1) (5), and to slow GE and induce conditioned flavor aversion, when infused intragastrically to rats (12). Furthermore, intragastric gavage of mice with bitter agonists increases c-fos expression in the nucleus tractus solitarius (NTS) of the brain stem. This effect is abolished following vagotomy, and, at least for the bitter tantant denatonium benzoate is dependent on CCK1 and Y2 receptors (14). However, although it has been reported that sham feeding with a bitter tantant slows GE in humans (50), it is not yet known whether intraintestinal bitter tantants slow human GE.

Bitter tasting is of further interest since a common genetic variant exists in humans, with some otherwise healthy individuals unable to report oral phenylthiocarbamide (PTC) as tasting bitter (22). Those bitter tantants that can safely be administered to the human gastrointestinal tract in adequate quantities (e.g., quinine and naringin) have also been reported to be rated less bitter by those who cannot taste PTC compared with those who can (6, 7). This raises the possibility that genotypic differences in responsiveness to bitter tantants might also exist in the gastrointestinal tract.

Taken together, these findings raise the possibility that the gastrointestinal tract may be able to sense and respond to sweetness and bitterness per se. In intact humans, it is not feasible to directly study gastrointestinal taste receptor activation; therefore, we determined whether nutrients that are perceived by the taste receptors in the tongue as being equally sweet or bitter would have equivalent effects on GE and perceptions of hunger and fullness. As the medulla houses the sensory (NTS) and motor nuclei responsible for relaying vagovagal enteric circuits, as well as signaling to higher centers, the determination of changes in GE permits an integrated measure of gut-brain signaling activity.

The aim of the current study was, therefore, to establish whether substances perceived to taste equally sweet or bitter by lingual taste receptors would have equivalent effects on gut-to-brain signaling responses in humans, i.e., whether ingested nutrients slow GE in a manner related to their taste. More specifically, our objectives were to evaluate the following hypotheses: 1) that, if the regulation of GE and appetite by the upper gastrointestinal tract is mediated by the detection of the sweetness or bitterness per se of ingested nutrients, then intragastric delivery of, “equisweet” or “equivetter” solutions would influence GE to a similar extent, independently of their chemical identity, and 2) that a glucose solution, made sweeter by the addition of the nonhexose sweet tantant saccharin, would slow GE more potently than either glucose or saccharin alone.

SUBJECTS AND METHODS

Twenty healthy subjects [10 for Study A, 12 for Study B (2 subjects participated in both arms of the study), 8 male, 12 female, mean age: 29 (range 19–60) yr, body mass index: 23.7 (1.3) kg/m²] were recruited from the staff and student population of Salford Royal NHS Foundation Trust. Subjects had no history of gastrointestinal symptoms or disease and were not on any medications known to affect gastrointestinal function, sensation, or appetite. Subjects were screened to determine whether they were “tasters” or “nontasters” of the bitter agonist PTC using PTC-impregnated blotting paper strips (Precision Laboratories, Norfolk, UK). Studies were conducted at 0900 after an overnight fast from 2200. Subjects provided informed, written consent before their enrolment in the study, approval for which had been granted by the Salford and Trafford Local Research Ethics Committee. All studies were carried out in accordance with the Declaration of Helsinki.

Materials

\(\text{D(+)Glucose, D(−)Fructose and quinine were purchased from Sigma-Aldrich (Gillingham, UK), saccharin from ACROS Organics (Fischer Scientific, Leicestershire, UK), aspartame from Konig and Wiegant (Dusseldorf, Germany), and naringin from Frutarom (Billingham, UK).}

Determining the “Sweetness” and “Bitterness” of the Solutions

Sweetness. Solutions were prepared using water containing sucrose, glucose, and fructose (0.2, 0.4, and 0.6 M), saccharin [50 (0.27 \(M\), 100 (0.55 \(M\)), 200 (1.1 \(M\)), 300 (1.6 \(M\)), and 400 (2.2 \(M\)) mg], and aspartame [100 (0.34 \(M\)), 200 (0.67 \(M\)), 300 (1.01 \(M\)), and 400 (1.36 \(M\)) mg] in 500 ml. A 1 M sucrose solution was prepared as a “benchmark” against which to rate the other solutions (45).

Subjects were familiarized with the 10-cm visual analog scale (VAS) questionnaire on which they would rate the sweetness, pleasantness, and intensity of the solutions. The VAS was anchored on the left with the term “not at all” and on the right with “extremely.” Subjects were instructed to taste and spit the water sample, which they were told was the least sweet that they would expect to encounter during the test and should receive a score of zero for sweetness. They were then asked to taste and spit the 1 M sucrose solution, which they were told was the sweetest that they would expect to encounter during the session and should receive a score of 10 (45). Subjects then tasted and scored each of the solutions listed above, in a single-blind, randomized fashion. This process was repeated for each subject, and the average of the two trials was used to construct a graph of sweetness ratings (Fig. 1A), which was used to determine the concentrations at which solutions of glucose, fructose, aspartame, and saccharin were equisweet. The resulting concentrations were equivalent to a score of 5 out of 10 for sweetness. Once these concentrations were determined, a group of subjects repeated this procedure to validate that the prepared solutions received a score of 5 out of 10 (Fig. 1B).

The solution of glucose + saccharin was constructed so that is was rated twice as sweet as the glucose or the saccharin alone.

Bitterness. Solutions consisting of water and 0.25, 0.5, and 1 mM of quinine or naringin were prepared. Ratings of bitterness were scored by 20 subjects following the protocol described above, and a graph of bitterness ratings was created (Fig. 2A), which was used to determine the concentrations at which solutions of quinine and naringin were equivitter. A line was drawn through the point at which the highest concentration of naringin tested (score 5 out of 10) was equivalent to that of quinine (concentrations at which we did not expect the bitter tantants to induce nausea). Because this fell slightly below the lowest concentration of quinine we had tested, we extrapolated a value of 0.198 mM quinine. This concentration of quinine was then tested in relation to 1 mM naringin to verify that this concentration was indeed equally bitter to that of naringin, which was demonstrated to be the case (Fig. 2B). There was no significant difference in ratings of bitterness between PTC tasters and nontasters.

Test Meals for GE Studies

Study A. The test meals consisted of 500 ml equisweet solutions of glucose (560 mosmol/kgH₂O, ~201 kcal), fructose (290 mosmol/kgH₂O, ~105 kcal), saccharin [50 mg (0.2 \(\mu\)M)], and aspartame [200 mg (0.67 \(\mu\)M)] and a twice as sweet solution consisting of glucose + saccharin [560 mosmol/kgH₂O and 50 mg (0.2 \(\mu\)M), respectively].
Study B. The test meals consisted of equibitter solutions of the bitter tastants naringin (1 mM) and quinine (0.198 mM).

In both studies, a meal of 500 ml water was also used to control for intragastric volume. The osmolality of the hexose test meals (mosmol/kgH2O) was confirmed using an osmometer (Cryoscopic osmometer OSMOMAT 030, Gonotec, Germany). This was not necessary for saccharin, aspartame, naringin, or quinine since none of these exceeded 1 mM, so can have no biologically relevant osmolality. All meals were infused at room temperature.

Assessment of GE

Each meal was labeled with 100 mg [13C]sodium acetate, used as a marker for GE (CK Gas Products). [13C]acetate is absorbed only after it is emptied from the stomach; hence, the rate of appearance of 13CO2 in the systemic circulation, and thereafter in expired air, closely corresponds to the rate of GE (43). End-expiratory breath samples were collected at 5-min intervals over a period of 45 min. On each occasion, the subject breathed through a mouthpiece to collect an end-expiratory breath sample in a 100-ml foil bag, which was then sealed with a plastic stopper and stored for later analysis. Breath samples were analyzed by nondispersive infrared spectroscopy using an isotope ratio mass spectrophotometer (IRIS; Wagner Analysen Technik, Bremen, Germany). This enabled evaluation of the isotopic composition of carbon in the expired CO2 by determination of the 13C-to-12C ratio in the sample (25). Although not a direct measure of GE, parameters obtained from 13C-labeled breath tests show an excellent correlation when compared with the “gold standard” scintigraphy method (26, 36). This technique therefore provides a simple, noninvasive, and repeatable biomarker of the activation state of gut-to-brain signaling.

Assessment of Hunger, Fullness, and Symptoms

Validated VAS questionnaires to assess perceptions of hunger and fullness, and any symptoms, e.g., bloating and nausea (34), were completed at 0, 5, 10, 15, 30, and 45 min. Each VAS evaluated a sensation on a 100-mm horizontal line, where 0 mm represented “sensation not felt at all” and 100 mm “sensation felt the greatest.” Subjects were asked to indicate how they were feeling at that particular time by placing a vertical mark on the line. Other perceptions, such as anxiety and drowsiness, were also assessed to distract from the main purpose of the questionnaire, but were not evaluated.

Effect of Sweetness on GE

Ten subjects each attended the laboratory on six occasions. Subjects were reclined in a relaxed semirecumbent (45°) position in an armchair or on a hospital bed, and a nasogastric feeding tube was positioned with its distal tip in the stomach. Before infusion of the test meal, a basal, end-expiratory, breath sample was collected. Immedi-
ately following this, subjects received a bolus intragastric infusion of the equisweet 500-ml test meals, consisting of glucose, fructose, saccharin, aspartame, water, or the “hyper-sweet” solution of glucose with added saccharin, in a single-blind, randomized fashion.

**Effects of Bitterness on GE**

Twelve subjects each attended the laboratory on three occasions. The study protocol described for Study A was repeated; however, subjects received intragastric infusions of equibitter solutions of quinine, naringin, or water.

**Data and Statistical Analysis**

GE data are displayed as raw values over time and areas under the curve (AUCs). AUCs of the GE data were calculated using the trapezoidal rule. VAS data are presented as change from baseline values, over time. GE data were analyzed comparing AUC values using repeated-measures ANOVA with treatment as a factor, and VAS scores were analyzed using repeated-measures ANOVA with time and treatment as factors. Post hoc paired comparisons, corrected for multiple comparisons using Bonferroni’s correction, were performed if ANOVAs revealed effects. Data are displayed as mean values (SE). Statistical significance was accepted at $P < 0.05$.

**RESULTS**

The study protocol was well tolerated. No subjects reported being able to orally taste the infused solutions. Intragastric administration of the sweet and bitter solutions was not associated with any gastrointestinal adverse effects. Two subjects were excluded from the analysis in Study A because they reported nausea on the water infusion day.

**Study A**

We were unable to demonstrate an effect of sweetness alone in the regulation of GE (Fig. 3, A and B). Although glucose slowed GE when compared with water ($P = 0.03$), equisweet solutions of fructose ($P = 0.15$) and the artificial sweeteners aspartame ($P = 0.24$) and saccharin ($P = 1.0$) did not.

A solution made to be twice as sweet as either glucose or saccharin alone had no additional effect on GE when compared with glucose alone ($P = 0.11$) (Fig. 4).

There was an effect of time, but no differential effect of any treatment, on perceptions of hunger or fullness (Fig. 5, A and B). Hunger decreased, and fullness increased, following all of the intragastric infusions (including the water), with scores returning toward baseline over the duration of the study.

**Study B**

We were unable to demonstrate an effect of bitterness alone on the rate of GE ($P = 0.97$) (Fig. 6, A and B). Naringin and quinine emptied from the stomach at the same rate as water. Upon dividing the subgroups post hoc, there was also no effect of PTC taster status on the rate of GE ($P = 0.46$), and no interaction between treatment and PTC taster status ($P = 0.91$) (Fig. 6C).

There was an effect of time, but no differential effect of treatment, on perceptions of hunger and fullness (Fig. 7, A and B). Hunger decreased, and fullness increased, following the intragastric infusions (including the water), with scores returning toward baseline over the duration of the study. Intragastric infusion of quinine and naringin did not induce nausea (Fig. 7C).

**DISCUSSION**

This study has demonstrated that, when administered directly in the human upper gut to bypass the activation of lingual taste receptors, equisweet tasting solutions do not have equivalent effects on the rate of GE. In our study, although the glucose solution slowed GE, neither the fructose, nor the artificial sweetener solutions, did so, despite tasting similarly sweet. Similarly, infusion of equibitter tastants in the stomach had no effect on GE or appetite in either PTC tasters or non-tasters. Therefore, the sweetness and bitterness taste per se of ingested nutrient solutions does not appear to be the principal recognition mechanism responsible for the regulation of GE or perceptions of hunger and fullness in humans.

Previous studies have established that the regulation of GE by hexose sugars is modulated by both the osmolality and the hexose composition of the ingested meal (9, 20, 30, 49). If a further factor by which hexose sugars were to signal to slow GE was according to their sweetness of taste, it would be predicted that fructose [which is ~2 times sweeter than glucose (3)] would empty from the stomach more slowly than an equiosmotic solution of glucose. Studies have demonstrated that fructose does empty from the stomach differently to glucose (9, 31). We have also recently demonstrated that a 250 mosmol/kgH$_2$O solution of fructose does indeed slow GE more potently than an equiosmotic solution of glucose, at least in some individuals (13). However, as the osmolality was increased to 500 mosmol/kgH$_2$O, both glucose and fructose slowed GE similarly, despite the fact that the differences in
sweetness remained (13). In the current study, to match for sweetness, the glucose meal was approximately two times the osmolality of the fructose meal, and this is likely to explain the differences in GE between the two hexose meals despite sweetness equivalence.

If the presence of sweetness taste per se in the upper gut played an important role in gut-to-brain signaling in humans, it would be expected that nonnutrient sweeteners, such as saccharin, sucralose, and aspartame, which activate T1R2/T1R3 receptor signaling in the tongue (33), would also slow GE. In a human L cell line, NCI-H716, the artificial sweetener sucralose has been reported to induce GLP-1 secretion (21). However, in the current study, neither saccharin nor aspartame had any effect on GE despite both being as sweet as the two hexose meals. These negative observations match the results of recent studies in animals (11) in which sucralose was found to have no effect on gastric inhibitory peptide or GLP-1 release, and a human study (27) that demonstrated that sucralose had no effect on GE or GLP-1 secretion. One potential criticism of the use of aspartame is that it may not have presented an adequate stimulus to the sweet receptors in the intestine since it would be digested in the gut to its constitutive, nonsweet, amino acids. However, saccharin, which is not metabolized, and only slowly absorbed from the small intestine, thus maintaining its sweet characteristics in the small intestinal lumen (39, 40), also showed no effect.

A role for sweet taste receptor-mediated pathways in glucose transport has also been reported (29), with receptor activation increasing expression of the enterocyte sodium-glucose co-transporter, SGLT1, an effect absent in both α-gustducin and...
T1R3-deficient mice. In perfused rat jejunum, sweet tastants (including non-nutrient sweeteners), also increase the expression of the glucose transporter GLUT 2 and the rate of glucose absorption (28). Such an increase in the rate of glucose absorption could influence the rate of GE by modulating signaling from the lumen. However, in the current study, the addition of saccharin to glucose, to increase the sweetness of the meal, had no additional delaying effect on GE when compared with glucose alone.

Administration of the bitter tastants, quinine and naringin, to the gastrointestinal tract at a concentration that tasted very bitter when placed in the mouth, also failed to affect GE. This is in contrast to studies in rats, in which intragastric infusion of denatonium benzoate (DB) at a concentration of 10 mM slowed GE (12), and both PTC and DB increased c-fos expression in the NTS (14). For our human studies, we were limited as to the amount, and type, of bitter tastant that we could administer in the gut, since we did not want to induce nausea or produce toxic effects; thus, the concentrations we administered were lower than those used in animal studies. The animal responses could therefore reflect either nonspecific effects of the tastants at the much higher doses used, or a species-specific difference.

Twenty five T2Rs are encoded by the human genome, and while the ligands for some are well characterized, e.g., the receptor for PTC (hT2R38) (41), the precise nature of the receptors to which quinine and naringin bind is unclassified, so it cannot be ruled out that the specific bitter receptor transcripts responsive to them are absent from the human small intestine. There is also evidence from animal studies that different bitter agonists have different effects on gut-to-brain signaling. For example, the bitter agonist DB has been reported to induce CCK secretion from enteroendocrine STC-1 cells (5). Also, in mice, gavage with PTC and DB increases c-fos expression in the NTS of the brain stem, an effect that is abolished following vagotomy, and for DB, but not PTC, is dependent on CCK1 and Y2 receptors (14). It is possible that both quinine and naringin, like PTC, have effects on T2Rs in the small intestinal lumen that are independent of CCK and PYY receptor activation; therefore, our physiological “read-out” of vagal afferent signaling (i.e., slowed GE) was unable to detect an effect. Nevertheless, our results demonstrate that the presence of an orally bitter tastant in the gut is, in itself, insufficient to stimulate this gastrointestinal response.

We found no effect of either bitter or sweet tastants on hunger or fullness, or nausea and bloating. The observed a decrease in hunger, and increase in fullness, following all of the intragastric infusions, including water, presumably because of the increased intragastric volume activating mechanosensitive afferents (44).

**Perspectives and Significance**

The results of this clinical physiological study do not support a role for the sweetness or bitterness taste of a meal in mediating the GE or hunger/fullness responses induced by ingested nutrients in humans. However, we cannot, of course, exclude the possibility that sweetness and bitterness per se may influence the release of gut peptides or other aspects of gastrointestinal function. It has been suggested that the activation of taste receptors in the gut would provide a means of targeting supplements for use in the treatment of obesity and type 2 diabetes by modulating gut-brain signaling (8). Our results, however, imply that the addition of artificial sweeteners or bitter agonists to food is unlikely to provide health benefits for the regulation of GE.

**ACKNOWLEDGMENTS**

We thank John Blundell and Dr. Eleanor Bryant of the Biopsychology Research Group, Institute of Psychological Sciences, University of Leeds, for assistance in developing the questionnaires and protocol for rating sweet and bitter taste and Annie Herbert (University of Manchester) for statistical advice.

**GRANTS**

T. Little was supported by an Overseas Clinical Research Postdoctoral Training Fellowship from the National Health and Medical Research Council.
of Australia. The study was supported by the Biotechnology and Biological Sciences Research Council Diet and Health Research Industry Club programme.

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