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Humans Can Taste Glucose Oligomers Independent of the hT1R2/hT1R3 Sweet Taste Receptor

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Abstract

It is widely accepted that humans can taste mono- and disaccharides as sweet substances, but they cannot taste longer chain oligo- and polysaccharides. From the evolutionary standpoint, the ability to taste starch or its oligomeric hydrolysis products would be highly adaptive, given their nutritional value. Here, we report that humans can taste glucose oligomer preparations (average degree of polymerization 7 and 14) without any other sensorial cues. The same human subjects could not taste the corresponding glucose polymer preparation (average degree of polymerization 44). When the sweet taste receptor was blocked by lactisole, a known sweet inhibitor, subjects could not detect sweet substances (glucose, maltose, and sucralose), but they could still detect the glucose oligomers. This suggests that glucose oligomer detection is independent of the hT1R2/hT1R3 sweet taste receptor. Human subjects described the taste of glucose oligomers as "starchy," while they describe sugars as "sweet." The dose–response function of glucose oligomer was also found to be indistinguishable from that of glucose on a molar basis.

Key words: carbohydrates, glucose oligomers, receptor, starchy, taste

Introduction

The primary function of taste is to identify substances that provide energy and/or electrolyte balance, while avoiding ingestion of toxic substances (Breslin 2013). Taste can also serve a metabolic function by preparing the body to assimilate ingested nutrients more effectively (Glendinning et al. 2015). Currently, there are 5 recognized taste categories in humans: sweet, sour, salty, bitter, and umami. Each taste quality is mediated by distinct transduction pathways expressed in subsets of taste receptor cells (Lindemann 1996; Adler et al. 2000). Specifically, sweet and umami tastes are detected by the G protein-coupled receptor (GPCR), T1R family, T1R2 + T1R3, and T1R1 + T1R3, respectively (Li et al. 2002; Zhao et al. 2003). Umami is detected by metabotropic glutamate receptors 1 (mGluR1) and 4 (mGluR4) as well (Yasumatsu et al. 2012). Bitter taste, on the other hand, is detected by GPCR T2R family (Chandrashekar et al. 2000).

© The Author 2016. Published by Oxford University Press. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com Sour and salty tastes are modulated by specialized membrane channels. For sour taste, acid sensing ion channels (ASICS; Ugawa et al. 1998), hyperpolarization-activated cyclic nucleotide-gated channels (HCNs; Stevens et al. 2001), and transient receptor potential channels (PKD2L1 and PKD1L 3; Ishimaru et al. 2006; Lopez Jimenez et al. 2006) facilitate its detection. For salty taste, epithelial sodium channel (ENaC) facilitates its detection (Heck et al. 1984; Kretz et al. 1999; Lin et al. 1999). The idea that there are only 5 taste categories has been challenged in recent years, however, by evidence that rodents can detect other ecologically important chemicals such as calcium (Tordoff 2001), fat (Fukuwatari et al. 1997), and starch hydrolysis products (Sclafani 2004) by the gustatory system.

Because starch is one of the primary sources of energy that enables the body to perform its function, its gustatory detection would be highly beneficial. However, the human gustatory detection of starch is thought unlikely because of its molecular structure and size. This reasoning does not account for the fact that during mastication salivary α -amylase hydrolyses the polymeric starch chains to shorter chain glucose-based saccharides, which are more likely to be detected by traditional receptor-based systems. Recently, we showed indirect evidence that humans can detect glucose oligomer and polymer mixtures commercially manufactured from starch (i.e., maltodextrins; Lapis et al. 2014). Results indicated that the intensity ratings were significantly correlated to one another but not to simple sugars (i.e., sucrose and glucose; Lapis et al. 2014). These data suggest that glucose oligomers and polymers can be detected through the gustatory system, independent of the sweet taste (Lapis et al. 2014). Additional support for this comes from animal studies wherein data suggest that rats were attracted to Polycose, a glucose oligomer and polymer mixture derived from starch, and preferred it over water, sucrose, and maltose especially at low equimolar concentrations (Feigin et al. 1987; Sclafani and Clyne 1987). Electrophysiological studies further confirmed that rats could differentiate between the tastes of Polycose and sucrose suggesting that they may have separate taste receptors for simple and glucose oligomer and polymer mixtures (Giza et al. 1991; Sako et al. 1994). Furthermore, combined genetic and behavioral studies showed that T1R2/T1R3 single or double knockout mice have severely impaired responses to sugars (e.g., glucose, maltose, sucrose, Na-saccharin) but near-normal responses to Polycose (Treesukosol et al. 2009, 2011; Zukerman et al. 2009; Treesukosol and Spector 2012).

In all of the studies previously mentioned, the stimuli used contain a broad mixture of glucose oligomers and polymers of varying chain lengths. For example, Polycose, a commercial maltodextrin derived from cornstarch, contains approximately 2% glucose, 7% maltose, and 91% glucose oligomers and polymers. Note that the definition of oligomers varies across fields of study; some consider those that contain 2-10 monomer units as oligomers (Rocklin and Pohl 1983; Sclafani 1987), whereas others count up to 20 as oligomers (Hughes and Johnson 1981). Consequently, it was not clear what substrates can facilitate the detection of glucose oligomers and/or polymers. Our lab, thus, recently fractionated a commercially available maltodextrin preparation based on differential solubility of its component saccharides in aqueous-ethanol solutions (Balto et al. 2016). The following groups of samples were produced: 1) glucose oligomers (i.e., Sample 1 [S1] and Sample 2 [S2] with average degree of polymerization, DP 7 and DP 14, respectively) and 2) glucose polymers (i.e., Sample 3 [S3] with average DP 44).

The objectives of this study were 1) to determine which glucose chain length ranges can be perceived through the gustatory system when stimuli were prepared at either equivalent % w/v concentrations or equivalent molar concentrations, 2) to investigate potential gustatory mechanism of glucose-based saccharides, 3) to establish the taste qualities of sugars and glucose-based saccharides, and 4) to establish the dose–response functions for sugars and glucose-based saccharides.

EXPERIMENT 1A. Taste discrimination of glucose oligomer and polymer stimuli prepared as equivalent % w/v solutions

In this experiment, the taste detection of glucose oligomers (S1 [average DP 7] and S2 [average DP 14]) and polymers (S3 [average DP 44]) was investigated. For detailed information regarding the samples, see Tables 1 and 2 in Balto et al. (2016). The composition of S1, S2, and S3 can be further seen in figures 3 and 4c, 3 and 4b, and 3 and 4d in Balto et al. (2016), respectively. An important

factor to consider when investigating taste detection of glucose oligomers and polymers is glucose oligomer/ polymer hydrolysis due to the presence of α -amylase in saliva. This hydrolysis results in a shift from the original DP profile of the test preparations, which could be a confounding factor when trying to identify the target substrates. Acarbose, a salivary amylase inhibitor (Clissold and Edwards 1988; Balfour and McTavish 1993; Martin and Montgomery 1996), was included in all test solutions to avoid this complication. In separate experiments, the efficacy of acarbose was determined by conducting in vitro salivary α -amylase hydrolyses of S1, S2, and S3, in the absence and presence of acarbose, using saliva collected from subjects with low, medium, and high activities (Supplementary Information). It was found that 5 mM acarbose was sufficient in inhibiting α -amylase, that is, no substantial amounts of hydrolysis products were produced when acarbose was present (Supplementary Figure 1). During pilot testing, 5 mM acarbose was also found to impart no detectable taste. In this experiment, the stimuli and blanks were thus prepared with 5 mM acarbose. It was expected that S1 would be most readily detected, followed by S2 and then S3; this expectation was based on suggestive findings in animal models that gustatory stimulation for glucose oligomers was greater than that for glucose polymers (Sclafani et al. 1987).

Materials and methods

Subjects

A total of 22 subjects (11 F, 11 M) between 18 and 45 years of age (mean = 25) were recruited from the Oregon State University campus and surrounding areas. Subjects who participated in the study were nonsmokers, not pregnant, not taking prescription pain medication or insulin, had no history of taste or smell loss or other oral disorders, had no oral lesions, canker sores, or piercings, and were without a history of food allergies. Prior to the test session, subjects were asked to comply with the following restrictions: 1) no dental work within 48h; 2) no alcohol consumption within 12h; 3) no consumption of foods and beverages that are acidic or caffeinated and/ or contain dairy within 4h; 4) no consumption of food or beverage of any kind except water within 1h; and 5) no use of any menthol-containing products within 1h prior to the scheduled sessions. In order to avoid deviations from normal α -amylase activity, subjects were also asked not to engage in physically demanding activity an hour before the test sessions. The experimental protocol was approved by the Oregon State University Institutional Review Board and complies with the Declaration of Helsinki for Medical Research. Subjects gave written informed consent and were paid to participate.

Stimuli

Aqueous solutions of 6% and 8% (w/v) S1, S2, and S3 were tested. These concentrations were chosen to elicit a taste sensation that was discriminable from water but was low enough such that there was no apparent viscosity. Deionized water served as blank stimuli. Target and blank stimuli were prepared with 5 mM acarbose. Stimuli were stored at 4–6 °C for a maximum of 5 days. All stimuli were brought to room temperature (20–22 °C) before providing to subjects.

Experimental protocol

Each subject was run through 2 test sessions, each on different days: 6% (w/v) glucose oligomer and polymer stimuli were presented in one session and 8% (w/v) stimuli were presented in the other. Half of the subjects tested 6% (w/v) first, the other half tested 8% (w/v) first. All testing was conducted on a one-on-one basis in a psychophysical testing room. Before each test session, subjects were verbally instructed

on the task they performed. To prevent olfactory input, subjects wore nose clips while performing the task. Subjects tasted 5 mL of each of the 3 stimuli, one at a time following a 3-s sip and spit procedure. After expectorating, the subject indicated which of the 3 stimuli was different by circling the 3-digit code of the corresponding sample on the ballot provided. Subjects were asked to rinse their mouth once with 37 °C water between each of the 3 samples within a set. They were also asked to rinse between each set of stimuli at least 3 times during a 1-min break. In both sessions, the subjects performed 3 sets of discrimination tests (i.e., triangle tests). The order of stimuli presentations was counterbalanced and randomized across subjects and sessions.

Data analysis

The number of correct identification for each stimulus was counted and was converted to d' values by consulting with d' table for triangle test (Ennis 1993). d' represents the detectability of the signal (stimulus) from the noise (blanks) and is a measure of separation of the noise and signal distributions. It is measured in terms of the number of standard deviations of the noise distribution. The d' analysis (Ennis 1993) was then used in order to determine significant discrimination.

Results

Table 1 shows the detectability (d' values) of S1, S2, and S3 prepared as equivalent % w/v solutions (Condition A). Results showed that subjects were able to significantly discriminate S1 and S2 against blanks but not S3 (P < 0.05). The detectability was highest for S1 followed by S2 at both concentrations. The d' values calculated for 8% w/v S1 was also higher than 6% w/v, while it was about the same for 6% and 8% w/v S2.

Table 1. Proportion correct and detectability (as d') of S1, S2, and S3

Condition	Concentration		Proportion correct		
			ď		
	% w/v	mM RE	S1	S2	\$3
A	6	_	0.82	0.55	0.32
	8	_	3.26 * 0.95	1.70* 0.59	0.00 0.27
			4.90*	1.93*	0.00
В	—	75	0.62 2.05*	0.58 1.86 *	0.38 0.77

Significance of bold values were provided. *P < 0.05.

Subjects performed sets of triangle tests by identifying an odd stimulus. Proportion correct for each stimulus was converted to *d'* (Ennis 1993). *d'* represents the detectability of the signal (stimulus) from the noise (blanks) and is a measure of separation of the noise and signal distributions. It is measured in terms of the number of standard deviations of the noise distribution. All stimuli were tested in the presence of 5 mM acarbose to inhibit salivary α -amylase. Conditions: (A) all stimuli were tested at equal 6% w/v, which is equivalent to 56, 26, and 8 mM RE for S1, S2, and S3, and 8% w/v, which is equivalent to 75, 35, and 11 mM RE for S1, S2, and S3, respectively. The stimuli were presented in 5 mL aliquots and were tasted by sip-and-split procedure; (B) all stimuli were 75 mM RE, which is equivalent to 8%, 17%, and 54% w/v for S1, S2, and S3, respectively. Tasteless methylcellulose was added to the stimuli in order to mask textural differences between stimuli.

*P < 0.05 by the d' analysis.

EXPERIMENT 1B. Taste discrimination of glucose oligomer and polymer stimuli prepared as equivalent mM solutions

Results of Experiment 1 provided an important insight on the potential substrate that humans can taste. However, comparisons across target stimuli may not be equitable since the stimuli were prepared based on % w/v. For example, S1 and S2, with relatively shorter saccharide chains, would have a higher molar concentration (i.e., more molecules present) at the same % w/v than S3, with relatively longer chains. This could potentially explain the discrimination of S1 and S2 but not S3. Consequently, in this experiment, we measured the detectability of the substrates at equal molar concentrations of reducing ends (RE). Each saccharide chain has 1 RE, thus moles RE is a direct measure of the number of molecules present. Substrate solutions for this experiment were 75 mM RE (i.e., equivalent to 8% w/v S1, 17% w/v S2, and 54% w/v S3). At these concentrations, however, the stimuli, in particular S2 and S3, had perceptible viscosities. We therefore prepared blank stimuli with matching viscosities from solutions of methylcellulose, a tasteless, nonthixotropic, viscogenic/gelling agent that is not a substrate of salivary α -amylase. The task was similar to the previous experiment except the stimuli were swabbed on the tip of the tongue to further minimize any textural cues. Again, it was expected that S1 would be most readily detected, followed by S2 and then S3 (Sclafani et al. 1987).

Materials and methods

Subjects

A total of 26 subjects (18 F, 8 M) between 18 and 33 years of age (mean = 25) were recruited from Oregon State University campus and surrounding areas. Inclusion criteria and restrictions were the same as in the previous experiment.

Stimuli

In this experiment, 75 mM REs S1, S2, and S3 were used as test stimuli. Seventy-five mM RE solutions of S1, S2, and S3 correspond to 8%, 17%, and 54% (w/v), respectively. In each case, the concentrations were based on RE due to the polydispersity of the glucose oligomer/polymer preparations (mmole RE/g stimuli are given in Balto et al. 2016). Blank samples were comprised of methylcellulose; methylcellulose solutions were prepared by heating solutions to 38-40 °C to aid dissolution. The viscosity of all solutions was determined using a Rapid Visco Analyzer 4500 (RVA; Perten Instruments) at 37 °C, 960 rpm, after 2 min of stirring. The viscosities of blank solutions were measured in triplicate, those of stimuli were measured without replicates due to limited sample. Viscosities of the 0.5%, 1.0%, and 3.8% (w/v) methylcellulose-containing blanks closely approximated those of the 75 mM RE S1, S2, and S3, respectively (Table 2). All stimuli and blanks were prepared with 5 mM acarbose. In order to further minimize any textural cues, the stimuli were swabbed on the tongue (i.e., approximately 0.20 mL was used to saturate a swab) instead of using the 5 mL sip and spit procedure. Samples were served at room temperature (20–22 °C).

Discrimination task

Before the test session, subjects were verbally instructed on the discrimination task they performed. With nose clips on, subjects extended their tongues out of the mouth and held it immobile between the lips. In a sequential manner, a set of 3 stimuli (1 target stimulus and 2 blanks) were applied by rolling a cotton swab saturated with the stimuli across the tip of the tongue 3 times. Subjects then retracted

Table 2. The viscosity (cP) of the test stimuli and their corresponding blank samples

Samples	Concentration (% w/v)	Viscosity (cP)	
S1	8.0	102	
Blank 1	0.5	108 ± 1	
S2	17.0	114	
Blank 2	1.0	116±1	
\$3	54.0	201	
Blank 3	3.8	206 ± 4	

All stimuli were equivalent to 75 mM RE. Blanks were prepared with aqueous solutions of methylcellulose as described in text. Viscosity was measured once for S1, S2, and S3 due to limited sample amount. Viscosity (\pm SE) was measured in triplicate for all the blanks.

their tongues without touching the roof or sides of the mouth and rinsed with 37 °C deionized water once before swabbing the next sample. After expectorating, subjects indicated which of the 3 stimuli was different by marking the ballot provided. Subjects were given 1 min break between sets of stimuli to rinse their mouths at least 3 times with 37 °C deionized water; they were allowed to remove nose clips during rinsing. Subjects performed a total of 3 sets of discrimination test. The presentation order of the sets and the stimuli within each set were randomized and counter-balanced across subjects.

Results

The detectability (d' values) of S1, S2, and S3 at equivalent mM concentrations is included in Table 1 (Condition B). As observed in Experiment 1A, subjects could significantly discriminate S1 and S2 against blanks in the absence of other sensory cues such as odor and texture, but not S3 (P < 0.05). Contrary to the prediction, however, the detection of S1 and S2 were about the same on a molar basis. Results suggest that humans can taste glucose oligomers but not polymers.

EXPERIMENT 2. Taste discrimination of sugars and glucose oligomers in the absence and presence of lactisole

Although our data show that humans can taste glucose oligomers, the transduction mechanism for its detection is unknown. Given that hT1R2/hT1R3 is responsible for the detection of sugars and other sweeteners (Li et al. 2002), it was of interest to investigate whether glucose oligomers are also detected through this receptor. To investigate this possibility, we used lactisole, a sweet taste blocker that binds to a pocket in the transmembrane region of hT1R3 and thus inhibits the sweet taste of sugars, proteins, and artificial sweeteners (Jiang et al. 2005). Lactisole itself is tasteless, but under some conditions it is known to cause sweet "water-taste" after it is rinsed away (Galindo-Cuspinera et al. 2006). Nevertheless, available data suggest that colder temperatures reduce the sweet "water-taste" (Green and Nachtigal 2013). During pilot testing, we confirmed that presenting the stimuli with lactisole and the rinse water at ~10 °C eliminated the sweet "water-taste." We then conducted a discrimination task using 3 sweeteners (glucose, maltose, sucralose) and 2 glucose oligomers (S1, S2), in the absence and presence of 1.4 mM lactisole. It was expected all stimuli would be discriminated in the absence of lactisole, while discriminability for sugars, but not glucose oligomers, would be compromised in the presence of lactisole.

Materials and methods

Subjects

A total of 25 subjects (18 F, 7M) between 18 and 41 years of age (mean = 25) were recruited from Oregon State University campus and surrounding areas. Inclusion criteria and restrictions were the same as in the previous experiments.

Stimuli

Two practice stimuli were provided: 1) 75 mM glucose and 2) 75 mM maltose. A total of 5 test stimuli were provided: 1) glucose, 2) maltose, 3) sucralose, 4) S1, and 5) S2. The sugars and glucose oligomers were prepared at 75 mM (mM RE for glucose oligomers). Sucralose was prepared at 0.025 mM, which was equi-intense as glucose. Water was used as blanks for sweeteners, while equiviscous methyl-cellulose blanks were used for glucose oligomers (Table 2). Acarbose (5 mM) was added to all target and blank stimuli. All target and blank stimuli were prepared in the absence and presence of 1.4 mM lactisole. Glucose solution was prepared at least the evening before the test session to allow for complete mutarotation of glucose tautomers (Pangborn and Gee 1961). All target and blank stimuli and rinse water were presented cold (~10 °C) to prevent sweet water taste elicited by lactisole.

Discrimination task

Subjects participated in 1 session. During practice, subjects were given 2 sets of 3 stimuli (1 target substrate, 2 blanks) using the swabbing technique described in the previous experiment. The subjects rinsed their mouths with cold water (~10 °C) between each stimulus. After tasting all 3 stimuli, the task was to identify which was different. The subjects were given a 1-min break between sets during which they rinsed their mouths with cold water (~10 °C) at least 3 times. Subjects proceeded to the test session only if they correctly identified both practice stimuli. Note that the practice/screening stimuli were provided to make sure that all subjects can detect the stimuli at the given concentration, which were presented at a low temperature (~10 °C). If they did not correctly identify 1 of the 2 target substrates, they were given another chance and the incorrectly identified target substrate was provided again. They only proceeded to the test session if they identified the target substrate correctly. Twenty-five out of the 33 subjects tested proceeded to the test session.

During the test, 5 stimuli were presented in 2 blocks: in the absence and presence of lactisole, giving a total of 10 sets of stimuli. The presentation order of blocks, sets of samples within the block, and samples within a set were pseudo-randomized across subjects. Pseudo-randomization was used because it was not practical to provide all possible presentation orders. The samples were provided in the same manner as in the practice session. Subjects performed a total of 10 sets of discrimination test during a session. Subjects were given 1 min break between sets of samples and 3 min break between blocks so that they can rinse their mouths with cold water (~10 °C) at least 3 times.

Results

Table 3 shows the detectability (*d'* values) of the stimuli in the absence and presence of lactisole. Results showed that all 5 target stimuli were detectable to about the same degree in the absence of lactisole. Lactisole, however, blocked the taste of glucose, maltose, and sucralose, that is, they could not be discriminated. In contrast, lactisole did not compromise the detectability of glucose oligomers. These finding suggests that a mechanism(s) other than the hT1R2/hT1R3 sweet receptor is responsible for the taste of glucose oligomers. Table 3. Proportion correct and detectability (as d') of the stimuli in the absence and presence of lactisole

	Proportion correct						
	<i>d'</i>						
	Glucose	Maltose	Sucralose	S1	S2		
Lactisole absent	0.56 1.77*	0.48 1.36*	0.56 1.77*	0.72 2.62*	0.48 1.36*		
Lactisole present	0.24 0	0.24 0	0.24 0	0.52 1.57*	0.44 1.14*		

Subjects performed sets of discrimination tasks. All stimuli were tested in the presence of 5 mM acarbose to inhibit salivary α -amylase. All stimuli were tested at 75 mM concentration except for sucralose (0.025 mM), which was tested at equi-intense concentration as glucose.

Significance of bold values were provided.

*P < 0.05 by the d' analysis.

EXPERIMENT 3. Determination of taste quality of glucose oligomers through a focus group discussion

If glucose oligomers were detected through a mechanism that is independent of the hT1R2/hT1R3 sweet receptor, it is suspected that the taste quality of glucose oligomers would be different from the sweet taste. During multiple studies we conducted, subjects always described the taste of glucose oligomer samples as "cereal-," "bread-," "cracker-," and "rice-like." The main objective of this current experiment, therefore, was to come up with one taste quality descriptor for glucose oligomers. In order to achieve this goal, we performed a focus group study.

Materials and methods

Subjects

A total of 7 subjects (6 F, 1 M; mean age = 28) participated in the study. They were recruited from Oregon State University campus and surrounding areas. The subjects who participated in this experiment also participated in the previous experiments; therefore, they had prior exposure to the glucose oligomer substrates.

Stimuli

Three equi-intense aqueous solutions of target stimuli were provided: 1) 100 mM sucrose, 2) 0.125 mM sucralose, and 3) 224 mM S2. These concentrations were determined through pilot testing. S2 was chosen over S1 as the test stimulus because S2 contains lower amounts of DP 3 (1.4% in S2 vs. 4.0% in S1; Balto et al. 2016), which was reported to activate the sweet taste receptors in animal models (Treesukosol et al. 2011). Importantly, the discriminability of S1 and S2 were about the same (see results of Experiment 2). Acarbose (5 mM) was added to all stimuli.

Focus group

Subjects participated in a 1 h group session held in a focus group room. Subjects were asked to taste the stimuli by swabbing across the tip of the tongue with nose clips on. They were unaware of the identity of the stimuli. They were encouraged to re-taste the stimuli as often as needed. During the initial round, each subject was asked to verbally describe the taste qualities perceived from each stimulus. The moderator kept notes on a white board so everyone could see other subjects' descriptors. Then, as a group, the subjects were asked to consolidate terms that were similar in meaning and they were eventually asked to come up with a one-word descriptor that best describes the taste of each stimulus. Subjects were given equal opportunity to express their perceptions at every stage of the group session, during which participants were selected in random order to share their opinions.

Results

As a group, subjects agreed that the taste perceived from aqueous solutions of sucrose was "sweet" like sugar water and sucralose was "sweet" like an artificial sweetener. They described the taste of glucose oligomers as "starchy" like a root vegetable, corn, bread, or pasta. Subjects also pointed out that the taste qualities of sucrose and sucralose were more similar to each other while the glucose oligomer was quite different from the other two.

EXPERIMENT 4. Establishing dose-response curves for sugars and glucose oligomers

In this experiment, it was of interest to know how the dose–response curves of glucose oligomers compare to that of sugars. Subjects were asked to rate the perceived intensities of different concentrations of sucrose, glucose, and glucose oligomers. The results from this experiment were expected to provide an idea of the differences in the amounts of simple sugars and glucose oligomers needed to elicit certain levels of responsiveness.

Materials and methods

Subjects

A total of 20 subjects (13 F, 7M; mean age = 25) participated in the study. They were recruited from Oregon State University campus and surrounding areas. Inclusion criteria and restrictions were same as in previous experiments.

Stimuli

Sucrose, glucose, and glucose oligomer (S2) were provided as stimuli. The concentrations were 45, 100, and 224 mM. Acarbose (5 mM) was added to all stimuli. Glucose was prepared at least the evening before the test session to allow for complete mutarotation of glucose tautomers (Pangborn and Gee 1961). All stimuli were presented at room temperature (20–22 °C).

Procedure

Subjects participated in only one session. Before the test, they were provided instructions and practice on the use of the general version of the Labeled Magnitude Scale (gLMS; Green et al. 1993, 1996; Bartoshuk et al. 2003). The scale was displayed on a computer monitor and subjects used a mouse to move a cursor along the scale to make their ratings. Subjects were provided the stimuli using a swabbing technique while wearing nose clips. After swabbing, the subjects were asked to perform a smacking motion twice with their mouth and then rate the perceived intensity of each stimulus, using the gLMS on a computer screen. Subjects were provided a total of 9 stimuli and they were given 1 min break between stimuli to rinse their mouth with 37 °C water at least 3 times. Subjects were also given a 3 min break after the 6th sample. The order of stimuli presentation was pseudorandomized across subjects.

Results

Figure 1 shows the dose–response functions for sucrose, glucose, and glucose oligomer based on molar and % w/v concentrations. On



Figure 1. Dose–response functions of glucose oligomer (S2), glucose, and sucrose based on (A) molar and (B) % W/V concentrations. The different filled symbols represent log means of intensity of the stimuli; the whiskers represent standard error. The *x*-axis indicates log concentration (M or % w/v) tested; the molar concentrations tested were 45, 100, and 224 mM for all 3 stimuli; the equivalent % w/v concentrations were 0.8%, 1.8%, and 4% for glucose, 1.5%, 3.4%, and 7.7% for sucrose, and 10.3%, 22.8%, and 51.1% for glucose oligomer. Left *y*-axis represents log perceived overall intensity. Right *y*-axis represents semantic labels of the gLMS: BD = barely detectable, W = weak, M = moderate. Subjects wore nose clips when performing the task. All samples were tested in the presence of 5mM acarbose to prevent salivary α -amylase hydrolysis of glucose oligomers.

a molar basis, the dose–response function for glucose oligomer was essentially indistinguishable from that for glucose. When plotted on a % w/v basis, the dose–response function for glucose oligomer was shifted to the right along the *x* axis. This difference illustrates the amounts of glucose oligomer needed on a weight basis to achieve the same level of perceived intensities compared to the simple sugars tested.

Discussion

Humans can taste glucose oligomers

The taste discriminability of glucose oligomers and polymers were measured based on both % w/v and mM RE. The results showed that subjects can discriminate glucose oligomers (S1 and S2) from water blanks but not glucose polymers (S3). It is important to note that in all experiments, the confounding effect of salivary α -amylase hydrolysis of the glucose oligomers and polymers was controlled for by using acarbose, an α -amylase inhibitor. Certainly, taste is not the only sensory cue that can be used for the discrimination of the glucose oligomers. The glucose oligomer and polymer samples evoke a very mild odor potentially due to the volatiles that were not removed during processing. Nevertheless, in all the current sensory studies, subjects wore nose clips to eliminate any olfactory cues. The glucose oligomer and polymer samples also impart notable texture depending on the size of molecule and concentration used. Accordingly, in Experiment 1B, the viscosities of glucose oligomers and polymers were matched with appropriate concentrations of methylcellulose, which were used as blanks in the discrimination task. With the combined use of methylcellulose as blanks and the swabbing technique used to deliver the stimuli (i.e., uses low volume of samples and imparts mechanical motion), the contribution of texture should have been minimized, if not eliminated. Overall, results suggest that in the absence of confounding effects of salivary α-amylase hydrolysis and other sensory cues (i.e., odor and texture), glucose oligomers, but not polymers, could be discriminated from blanks through the gustatory system.

Target substrates for taste detection

The present results showed that the detectability of the glucose oligomers, S1 with average DP 7 and S2 with average DP 14 were about the same on a molar basis. This finding is partly consistent with those obtained with rats, which suggested that among the saccharides tested (i.e., glucose, maltose, maltotriose, Polycose, glucose oligomers [average DP 6], glucose polymers [average DP 43]), the

optimum stimulus for detection was glucose oligomer preparation with an average DP of 6 (Sclafani et al. 1987). There is a discrepancy, however, in whether glucose polymers can be tasted; our findings indicate that humans cannot taste glucose polymers with an average DP of 44, while rodents can detect glucose polymers with an average DP of 43 (Sclafani et al. 1987). In that study, however, the salivary α -amylase activity was not controlled for, so it was possible that some hydrolysis products produced in the oral cavity confounded their results. Future studies that explore the lower or upper limit of glucose chain lengths that could serve as target substrates must be conducted to further elucidate the mechanisms that support glucose oligomer taste detection.

When psychophysical functions were measured, the function for glucose oligomers was essentially indistinguishable from that for glucose on a molar basis. Note, however, that methylcellulose was not added to the stimuli in this experiment, although the potential contribution of viscosity was minimized by employing a swabbing technique. Nevertheless, it is possible that the apparent equal intensity ratings of glucose and glucose oligomer may have occurred in part due to the possibility that the taste of glucose was more intense than glucose oligomer whereas glucose oligomer was more viscous than glucose. With this caveat, our current results highlight the differences in the amount of simpler sugars and glucose oligomers needed to prepare the aqueous solutions to generate the same level of responsiveness.

Taste discrimination of glucose oligomers is not through the sweet taste receptors

At present, it is widely recognized that when it comes to carbohydrates, sugars are the only ones that can be tasted through the T1R2/T1R3 taste receptors. The T1R2 and T1R3 are members of the large family of GPCRs that combine to form a heterodimer and are involved in the perception of sweetness (Nelson et al. 2001; Fernstrom et al. 2012). A relevant question is whether or not the glucose oligomer preparations are detected through the same set of taste receptors that detect sugars. To test this, lactisole was used as a T1R2/T1R3 taste receptor "blocker" (sweetness inhibitor; Jiang et al. 2005). Lactisole is tasteless by itself, however, it causes sweet "water-taste" that is, when lactisole is rinsed away, it is removed from the sweet receptor, which then activates the cell and results to sweet perception of pure water (Galindo-Cuspinera and Breslin 2006; Galindo-Cuspinera et al. 2006). This could potentially be a confounding factor when determining the discriminability of glucose oligomers in the presence of lactisole. This confounding effect was eliminated when stimuli and rinse water were presented to subjects at colder temperature (~10 °C). Results of Experiment 2 showed that while the taste of all stimuli (glucose, maltose, sucralose, S1, and S2) can be discriminated against blanks in the absence of lactisole, only S1 and S2 can be discriminated in the presence of lactisole. Note that other confounding factors (i.e., salivary amylase and other sensory cues, odor, and texture) were again controlled for in this experiment. This finding is consistent with that of animal models wherein combined genetic and behavioral studies showed that T1R2/T1R3 single or double knockout mice had severely impaired responses to sugars (e.g., glucose, maltose, sucrose, Na-saccharin) but near-normal responses to Polycose, a glucose oligomer and polymer mixture (Treesukosol et al. 2009, 2011; Zukerman et al. 2009; Treesukosol and Spector 2012). Results suggest that mechanisms other than the hT1R2/hT1R3 are responsible for the detection of glucose oligomers. In fact, results of our focus group showed that glucose oligomers were described as "starchy" instead of "sweet" like the other sweet substances tested (i.e., sucrose and sucralose).

Potential mechanisms of glucose oligomer detection

First, it is possible that T1R-independent sugar or sweet sensing pathways mediate the detection of glucose oligomers. It has been found that glucose transporters (GLUTs), sodium-glucose co-transporter 1 (SGLT1), and ATP-gated K⁺ (K_{ATP}) metabolic sensors are co-expressed in the taste cells with T1r3 and function to detect sugars in mice (Merigo et al. 2011; Yee et al. 2011; Margolskee et al. 2015). These particular T1r-independent pathways would, however, only explain responses to monosaccharides (e.g., glucose and fructose) but not disaccharides (e.g., maltose and sucrose), which are not transported by these sensors (Margolskee et al. 2015). If glucose oligomers utilize this transduction pathway, they need to be hydrolyzed to monosaccharides by enzymes involved in carbohydrate digestion. It has been shown that intestinal enzymes such as α -amylase and maltase-glucoamylase, which hydrolyze glucose oligomers to glucose, are expressed in taste tissues and cells (Merigo et al. 2009; Margolskee et al. 2015). Thus, it is plausible that a T1Rindependent sugar or sweet sensing pathway mediates detection of glucose oligomers via their hydrolysis to glucose. Interestingly, the T1r-independent pathways do not appear to generate salient taste sensation in mice, although it triggers gustatory nerve responses (Glendinning et al. 2015). A more critical function of the known and unknown T1r-independent taste transduction pathways in mice had been associated with metabolic responses that prepare the body for digestion and nutrient assimilation (e.g., cephalic phase insulin release [CPIR] and enhancing glucose tolerance; Glendinning et al. 2015; Schier and Spector 2016). It was also reported that T1r3 KO mice exhibited CPIR following oral administration of glucose and sucrose but they did not alter the behavior of mice (i.e., they did not lick sugar more than water) suggesting that the T1r-independent pathway for sugars did not generate a salient taste sensation (Glendinning et al. 2015).

If a T1R-independent pathway was involved in conscious perception of sugars, one would expect that glucose and maltose should be perceived whether hT1R2/hT1R3 sweet receptor was blocked or not. In our study, however, both glucose and maltose were not discriminated from blanks in the presence of lactisole, while glucose oligomers still were. Note, however, that in all of the experiments we conducted, all stimuli were expectorated, which limited the stimulation of the posterior field of taste buds. In other words, if the stimuli were swallowed, the general detectability might have been higher and thus it is possible that T1R-independent detection of glucose and maltose could have occurred even in the presence of lactisole. Regardless, the observations in our current study lead to the conclusion that the T1R-independent sugar sensing pathway is not, at least, the primary mechanism in the salient taste perception of glucose oligomers.

Alternatively, a novel taste receptor might be involved in the transduction mechanism used to detect glucose oligomers. This has been proposed (Sclafani 2004) but not yet identified. If such a novel taste receptor exists, it must be capable of generating a salient taste perception and the taste quality should be different from other taste categories (e.g., sweet taste). Results from our focus group confirmed this idea as subjects described sucrose, sucralose, and glucose oligomer as "sweet" like sugar water, "sweet" like an artificial sweetener, and "starchy" like a root vegetable, corn, bread, or pasta, respectively.

Summary

In light of our current data, this study provides the first direct demonstration that humans can taste glucose oligomers (average DP 7 and 14) through the gustatory system without the confounding effect of salivary α -amylase and other sensory cues (i.e., odor and texture). The detection of glucose oligomers seemed to be independent of the hT1R2/hT1R3 sweet taste receptor. In addition, the taste quality of glucose oligomers is in fact different from that of sugars. Importantly, while glucose oligomers are not ubiquitous in nature as a food source, they can be produced in the mouth during oral digestion of starch by way of salivary α -amylase hydrolysis (Lapis TJ, Penner MH, Lim J, unpublished data). If an independent mechanism for glucose oligomers exists, its main function is presumably to identify and signal the body of incoming starch, a substance that is an important source of energy.

Supplementary material

Supplementary materials can be found at http://www.chemse. oxfordjournals.org/

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Conflict of interest

The authors declare no competing financial interests.

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