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RESEARCH ARTICLE

Correlation between in vitro binding activity of sweeteners to cloned human sweet taste receptor and sensory evaluation

Yoonha Choi[1] · John A. Manthey[2] · Tai Hyun Park[3,4] · Yeon Kyung Cha[3] · Yang Kim[5] · Yuri Kim[1]

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Abstract The human sweet taste receptor is a TAS1R2/TAS1R3 heterodimer. To investigate the correlation between the in vitro affinity of sweeteners with stably expressed human sweet taste receptor in HEK-293 cells and human sensory evaluation, the receptor-ligand activity of bulk (sucrose, D-fructose, and allulose) and high-intensity sweeteners (saccharin, rebaudioside A, rebaudioside M, and neohesperidin dihydrochalcone) was compared by analyzing the Ca^{2+} release. The relative potency of the sweeteners was identified over a wide concentration range for EC_{50}s. Relative to sucrose, bulk sweeteners showed similar concentration ranges and potency, whereas high-intensity sweeteners exhibited lower concentration ranges and higher potency. The log of the calculated EC_{50} of each sweetener relative to sucrose by the in vitro affinity assay was positively correlated ($r = 0.9943$) with the molar relative sweetness reported in the previous literatures. These results suggested a good correlation between the in vitro activity assay of sweeteners and human sensory evaluation.

Keywords Sweet taste receptor · Sensory evaluation · In vitro activity assay · Sweetener · Correlation

✉ Yuri Kim
yuri.kim@ewha.ac.kr

Yoonha Choi
ch19864@naver.com

John A. Manthey
john.manthey@usda.gov

Tai Hyun Park
thpark@snu.ac.kr

Yeon Kyung Cha
ykchacha12@snu.ac.kr

Yang Kim
yankim@snu.ac.kr

[1] Department of Nutritional Science and Food Management, Ewha Womans University, Seoul 03760, Republic of Korea

[2] Agricultural Research Service, U.S. Horticultural Research Lab, U. S. Dept. of Agriculture, 2001 South Rock Road, Fort Pierce FL34945, USA

[3] Interdisciplinary Program for Bioengineering, Seoul National University, Seoul 08826, Republic of Korea

[4] School of Chemical and Biological Engineering, and Institute of Chemical Processes, Seoul National University, Seoul 08826, Republic of Korea

[5] Center for Food & Bioconvergence, Seoul National University, Seoul 08826, Republic of Korea

Introduction

Mammals can distinguish five basic tastes (bitter, umami, sour, salty, and sweet), which provide sensory information for evaluating taste of food (Adler et al., 2000). Taste receptor cells are distributed across different papillae of the tongue and palate epithelium. These cells are responsible for taste sensing (Chandrashekar et al., 2006). Among the five tastes, the detection of bitter, umami, and sweet taste is mediated by interactions between various ligands and specific types of G-protein-coupled receptors (GPCRs) belonging to the superfamily of transmembrane-bound receptors (Lagerström and Schiöth, 2008).

Sweet taste sensing is mediated by heterodimeric GPCRs, TAS1R2 and TAS1R3 subunits, confirmed through a cell-based form comprising TAS1R2 and TAS1R3 (Li et al., 2002b; Nelson et al., 2001; Xu et al., 2004; Zhao et al., 2003). Sweet-tasting compounds, including natural and artificial sweeteners, amino acids, and even sweet proteins, elicit sweetness by binding to a

specific binding site on the TAS1R2/TAS1R3 heterodimeric sweet taste receptor (DuBois, 2016; Fernstrom et al., 2012). Binding of the ligand to the GPCR induces a conformational change in the sweet taste receptor from its inactive state to its active state which causes the separation of G_{α} subunit from $G_{\beta\gamma}$ and each G_{α} and $G_{\beta\gamma}$ interact with other intracellular processes to continue the signal transduction cascade (Li et al., 2002a). The GPCR signaling pathways activate a phosphoinositide pathway that elevates cytoplasmic Ca^{2+} and depolarizes the membrane via a cation channel, TRPM5 (Chaudhari and Roper, 2010).

Sweeteners are categorized into bulk sweeteners and high-intensity sweeteners depending on the degree of sweetness. Bulk sweeteners are generally carbohydrates that supply energy (calories) and are consumed in comparable amounts to sugar because of their similar sweetness to sugar. In contrast, high-intensity sweeteners have sweetness but almost zero calories, and so are consumed in low quantities because of their high sweetness intensity compared with sugar (Yebra-Biurrun, 2013). Bulk sweeteners include the monosaccharides, such as allulose, erythritol, fructose, glucose, mannitol, sorbitol, and xylitol and disaccharides, such as isomalt, lactitol, maltitol, and sucrose. High-intensity sweeteners include acesulfame K, aspartame, cyclamate, neotame, sucralose, saccharin, rebaudioside A (Reb A), rebaudioside M (Reb M), thaumatin, and neohesperidin dihydrochalcone (NHDC). Monosaccharides and disaccharides are mostly caloric sweeteners, whereas sugar alcohols or polyols among bulk sweeteners are low calorie sweeteners which are incompletely absorbed in the intestine by passive diffusion. Moreover, generally available high-intensity sweeteners or their metabolites are quickly absorbed into the gastrointestinal tract and are excreted (Yebra-Biurrun, 2013).

Sensory evaluation analysis is the easiest way to analyze the quality of taste and sweetness in developing new sweet-tasting molecules low in calories. In addition to the extensive time and cost required in sensory training and evaluation, each individual's diverse preference and sensitivity levels increase the subjectivity of the evaluation and cause sensory fatigue problems during the sensory evaluation (Njoman et al., 2017). The availability of in vitro activity assays with cloned sweet receptors is a useful alternative to reduce these various limitations of sensory evaluation. For example, a high throughput screening assay was developed for the human sweet taste receptor to correlate the in vitro receptor activity and human sensory taste tests (Li and Servant, 2008).

The objective of this study is to analyze the sweet taste receptor activity in response to a variety of sweeteners and investigate the correlation between in vitro activity of the cloned human sweet taste receptor with human sensory evaluation in the context of bulk (mono-, and di-saccharide) and high-intensity sweeteners.

Materials and methods

Reagents

Sucrose, D-fructose, and saccharin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Allulose, Reb A, and Reb M were kindly gifted by CJ Cheiljedang Corp., Korea. NHDC was kindly gifted by Dr. Manthey from U.S. Horticultural Research Lab. All sweeteners were kept in an auto-desiccator (Sanpla Dry Keeper, Sanplatec Corp., Osaka, Japan). Sucrose, D-fructose, allulose, and saccharin were solubilized at a range of concentrations in Hank's Balance Salt Solution (HBSS; Gibco, Paisley, Scotland, UK) supplemented with 20 mM HEPES. Reb A, Reb M, and NHDC were solubilized at a range of concentrations in 5% ethanol.

Cell culture

The human embryonic kidney-293 (HEK-293) cell line was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). HEK-293 cells were cultured in low-glucose Dulbecco's Modified Eagle's Medium (DMEM, Welgene, Daegu, Korea) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA), 1% penicillin (100 U/mL), and 100 µg/mL streptomycin (Invitrogen, Carlsbad, CA, USA). Cells were maintained in an incubator at 37 °C with 5% CO_2.

Construction of HEK-293 cells expressing TAS1R2 and TAS1R3 and transfection to HEK-293 cells

Recombinant plasmid constructs, pCMV6-Entry containing TAS1R3 and pCMV6-Entry containing TAS1R2 fused with green fluorescent protein (GFP) and Gα15 gustducin, were constructed and kindly gifted by Dr. Tai Hyun Park, Seoul National University, Korea. HEK-293 cells were transfected with pCMV6-Entry containing TAS1R3 using Lipofectamine™ 3000 (Invitrogen) according to the manufacturer's protocols. After 48 h, cells were transferred to medium containing 1 mg/mL G-418 (Gibco) for selection. After 1 month, G-418 resistant cell colonies were separately picked up and cultured, then used for measuring the intracellular calcium influx to sweeteners. The expression of hTAS1R3 in the cell membrane was confirmed by Western blot analysis. The HEK-293 cell line stably expressing TAS1R3 was transfected with pCMV6-Entry containing TAS1R2 fused with GFP using Lipofectamine™ 3000 (Invitrogen). After transfection for 48 h,

the expression of hTAS1R2-GFP was confirmed by monitoring the green fluorescence under a fluorescence microscope (Nikon Instruments Co., Ltd., Tokyo, Japan).

Western blot assay

All samples were isolated using buffer A [150 mM NaCl (Samchun, Seoul, Korea), 50 mM HEPES (Biosesang, Gyeonggi, Korea) (pH 7.4), 25 μg/mL digitonin (Sigma-Aldrich), 1 M hexylene glycol (Sigma-Aldrich), and 1% (v/v) protease inhibitor cocktail (Sigma-Aldrich)] and buffer B [150 mM NaCl (Samchun), 50 mM HEPES (Biosesang, Gyeonggi, Korea) (pH 7.4), 1% (v/v) Igepal (Sigma-Aldrich), 1 M hexylene glycol (Sigma-Aldrich), and 1% (v/v) protease inhibitor cocktail (Sigma-Aldrich)] to extract cytoplasmic and membrane fractions, respectively (Baghirova et al., 2015). Equal amount of each fraction was separated in 6% sodium dodecyl sulfate (SDS) polyacrylamide gels. Following electrophoresis, separated proteins were transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA), and blocked with 5% skim milk in Tris-buffered saline/Tween 20 (TBS-T). The blocked membranes were incubated with primary antibodies recognizing a TAS1R3 (Abcam, Cambridge, MA, USA) and α-tubulin (Sigma-Aldrich) at 4 °C overnight. The membranes were washed several times with TBS-T, followed by the addition of anti-rabbit or anti-mouse secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After 1 h, an enhanced chemiluminescence (ECL) solution (Visualprotein, Taipei, Taiwan) detection system was used to visualize bound antibodies.

Calcium mobilization assay

For calcium signaling analysis, HEK-293 cells stably expressing TAS1R3 were seeded at 50,000 cells/well into 96-well, clear-bottomed black plates (Corning, Inc., Corning, NY, USA) coated with poly-D-lysine (Sigma-Aldrich). The cells were co-transfected with TAS1R2 and Gα15 gustducin using Lipofectamine™ 3000 (Invitrogen). After 24 h, the medium was replaced with fresh medium, and the co-transfected cells were recovered. After an additional 24 h, the cells were assayed using the Fura-2 QBT fluorescence-based-calcium indicator kit (Fura-2 QBT™ Calcium Kit, Molecular Devices, Sunnyvale, CA, USA) according to detect calcium mobilization. Activation of sweet taste receptors in the cells was detected by measuring the ratio of the fluorescent intensities immediately after applying various sweet taste receptor agonists. The signals were measured using a Flex Station III fluorescence plate reader (Molecular Devices) at 510 nm, following excitation at 340 nm for the Ca^{2+}-bound Fura-2 dye, and 380 nm for unbound Ca^{2+}, respectively ($\Delta F_{340\ nm}/F_{380\ nm}$ ratio, abbreviated to $\Delta F/F$)).

Calculation formulas

The relative sweetness (RS) value is transformed into molar relative sweetness (MRS), because the effects of the potency of each compound at the molecular level can be related to the receptor-ligand binding affinity (Bassoli et al., 2008). Hence, to compare the correlation between the in vitro assay for sweet taste activity and human sensory evaluation, the RS of each compound was recalculated on a molar basis by the formula: MRS = RS × [molecular weight (compound)/molecular weight (sucrose)] (Bassoli et al., 2008). The relative potency of each compounds to sucrose was calculated as follows: Relative potency = EC_{50} in affinity assay (sucrose)/EC_{50} in affinity assay (compound) (Li and Servant, 2008).

Statistical analysis

All experimental results were expressed as the mean ± S.E. of the ΔF/F value of three independent experiments. Data were analyzed by using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Eight different concentrations of the sweet ligands were analyzed to construct the dose-dependent curve. Activities were normalized to the response baseline fluorescence level of each compound. EC_{50} values were determined by a nonlinear regression algorithm. Pearson's correlation coefficient (r) was determined between the mean MRS and relative potency (EC_{50}) in the assay of sucrose.

Results and discussion

Heterodimeric human sweet taste receptors

The expression of hTAS1R3 in the stable HEK-293 cell line was confirmed from the membrane fractions of TAS1R3-stable cells by Western blot analysis (Fig. 1A). After constructing the stable cell line expressing hTAS1R3, the TAS1R3 stable cells were transiently transfected with the hTAS1R2 gene fused with GFP to induce the heterodimer of hTAS1R2 and hTAS1R3. We confirmed the expression of hTAS1R2 in the TAS1R3 stable cells by fluorescence microscopy (Fig. 1B). Green fluorescence was observed from the TAS1R3-stable cell line transfected with hTAS1R2-GFP. These results indicate that the human sweet taste receptor composed of hTAS1R2 and hTAS1R3 was successfully co-expressed in HEK-293 cells.

G-protein subunit Gα15 gustducin is expressed with TAS1R2 and TAS1R3, as the key components of the

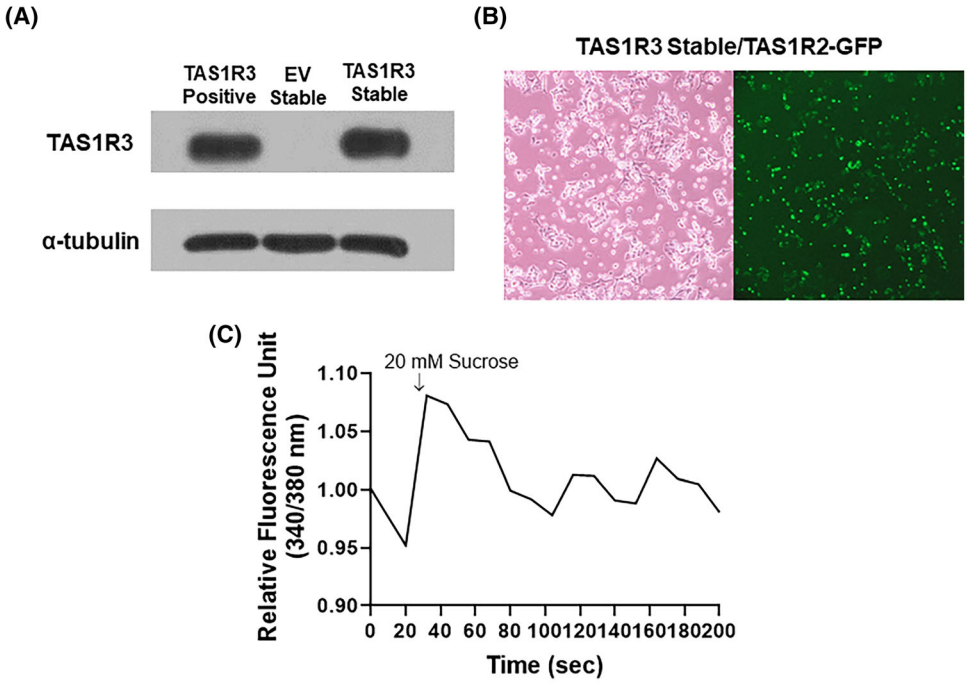

Fig. 1 Heterodimeric human sweet taste receptors. (**A**) Expressed hTAS1R3 was detected using Western blot in HEK-293 cells expressing TAS1R3. α-Tubulin served as the loading control. (**B**) Fluorescence image of GFP-tagged hTAS1R2 co-expressed in HEK-293 cells stably expressing TAS1R3. (**C**) Ca^{2+} signaling analysis in HEK-293 cells expressing TAS1R2/TAS1R3/Gα15 gustducin after adding 20 mM sucrose. EV, empty vector control

sweet-taste transduction cascade (Nelson et al., 2001; Xu et al., 2004). To investigate whether these taste receptors were functional, Ca^{2+} influx was measured in HEK-293 cells expressing the human sweet taste receptor by the Fura-2 calcium indicator dye assay after injection of sucrose (Fig. 1C). Addition of sucrose (20 mM) to TAS1R2, TAS1R3, and Gα15 gustducin expressing cells induced an immediate increase in ΔF/F. In previous studies, neither HEK293-Ga15 cells, which did not express the human taste receptors, nor monomeric receptor expressing cells exhibited any functional response to various sweeteners in the calcium signal analysis. Co-transfection of TAS1R2, TAS1R3, and Gα15 gustducin is required for agonist-selective, receptor-specific interaction of the sweet taste receptors, as each subunit alone does not show functional activity (Ahn et al., 2016; Bassoli et al., 2008). Ahn et al. (2016) constructed a duplex bioelectronic tongue functionalized with heterodimeric human umami taste (TAS1R1/TAS1R3) and sweet taste receptor (TAS1R2/TAS1R3) nanovesicles (Ahn et al., 2016). In the present study, it was confirmed that HEK-293 cells did not exhibit sweet taste receptor activity in response to 20 mM sucrose when only one subunit (TAS1R2, TAS1R3, or Gα15 gustducin) was transfected individually (data not shown). These results indicated that this calcium mobilization was not due to the background and the human sweet taste receptor by co-expression of TAS1R2, TAS1R3, and Gα15 gustducin was an efficient model in response to binding of sucrose.

Dose-dependent analysis of various concentrations of sweeteners interacting with the human sweet taste receptor

HEK-293 cells expressing the human sweet taste receptor were stimulated with increasing concentrations of various sweet ligands (Fig. 2). Sweet taste sensitivity was normalized to the response of sweeteners between 0 and 100% concentration. The percentage of normalized response increased as the concentration of the sweetener increased and was saturated at a high concentration of each sweetener. Consistent with a previous study (Yebra-Biurrun, 2013), bulk sweeteners and high-intensity sweeteners displayed a similar and much higher degree of sweetness (by about a few hundred times) than sugar, respectively. The concentration ranges for the percentage of normalized response differed considerably between bulk sweeteners and high-intensity sweeteners. Among the bulk sweeteners, monosaccharides (D-fructose and allulose) showed similar concentration ranges for the percentage of normalized response of sucrose (disaccharide). In comparison, four high-intensity sweeteners (saccharin, Reb A, Reb M, and NHDC) had much lower concentration ranges for the percentage of normalized response of sucrose. This difference in the degree of sweetness distinguishes bulk sweeteners from high-intensity sweeteners which was confirmed through dose-dependent analysis.

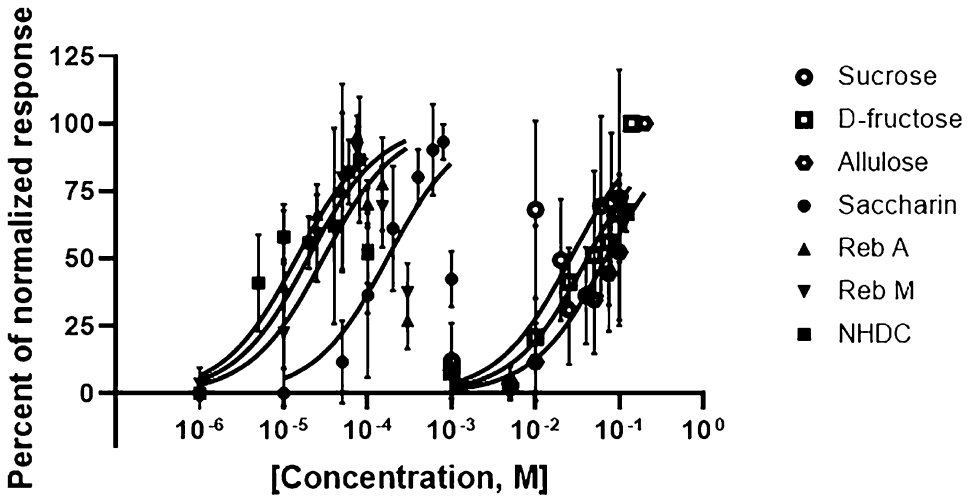

Fig. 2 Dose-dependent analysis of various concentrations of sweeteners interacting with the human sweet taste receptor. The percentage of normalized response of sweeteners was performed at eight different concentrations with 100 times concentration difference in each range in HEK-293 cells expressing the human sweet taste receptors. Dose-dependent response of bulk sweeteners (D-fructose and allulose) and high-intensity sweeteners (saccharin, Reb A, Reb M, and NHDC) was confirmed. NHDC, neohesperidin dihydrochalcone; Reb, rebaudioside

Correlation analysis between the potency of sweeteners relative to sucrose by in vitro affinity assay and the mean MRS values

The relative potency of sweeteners to sucrose (EC_{50}) by the affinity assay and the relative sweetness (RS) to sucrose by human sensory evaluation from previous studies are summarized in Table 1. The bulk sweeteners (monosaccharides, D-fructose and allulose) were determined as less potent sweetener (EC_{50}s > 1 mM), displaying a similar potency of sweetness to sucrose (disaccharide). The high-intensity sweeteners (saccharin, Reb A, Reb M, and NHDC) were found to be more potent sweeteners (with EC_{50}s < 1 mM), showing low potencies relative to sucrose (disaccharide).

The RS of D-fructose was 1.17 to 1.80, which is higher than the RS of sucrose, 1.00 (Colonna et al., 2000; Featherston, 2015; Parker et al., 2010; Tiefenbache, 2017). The MRS value for D-fructose (relative potency of 0.63) ranged from 0.62 to 0.95. Allulose had an RS of 0.70, which is lower than that of sucrose, RS of 1.00 (Chung et al., 2012; Tiefenbacher, 2017). The MRS value of allulose (0.37) was close to the relative potency of allulose relative to sucrose, 0.38.

Saccharin is 200–700 times sweeter than sucrose (Colonna et al., 2000; Featherstone, 2015; Parker et al., 2010; Tiefenbacher, 2017; Yebra-Biurrun, 2013). The MRS value of saccharin ranged from 107.03 to 374.61, considering the relative potency of saccharin (151.52). Reb A is 100–300 times sweeter than sucrose (Carakostas et al., 2008; Goyal et al., 2010; Kemp and Lindley, 2009). The relative potency of Reb A (1,338.14) was much higher and outside the MRS range of Reb A (282.5–847.51). However, another rebaudioside, Reb M, which is reported to be 200–350 times sweeter than sucrose (Prakash et al., 2014), showed an MRS range from 754.32 to 1,320.05 and relative potency of 880.16. Generally, Reb A is the sweetest analog among steviol glycosides, but it has a high relative bitter taste (Hellfritsch et al., 2012).

In the human sensory evaluation, the perception of bitterness affects the relative recognition of sweetness. Saccharin is an agonist for both sweet and bitter receptors, and cyclamate is an agonist for the sweet receptor only and an antagonist for the bitter receptor. It is reported that sweetness is enhanced synergistically when saccharin and cyclamate are mixed. This increased sweetness recognition and reduced bitter off-taste are due to inhibition of the bitter taste receptor activity of cyclamate (Behrens et al., 2017). Reb M is produced by enzymatic glycosylation of Reb A, which compensates for the bitterness of Reb A. Reb M has different types of chemical structure, which may have other effects on receptor-ligand binding (Spakman, 2015). Conformational change occurs when the TAS1R2/TAS1R3 heterodimer and ligand bind together. The TAS1R2/TAS1R3 heterodimer becomes an active form of closed–open, through the three-dimensional structure. This can be predicted by which Reb A is formed closer than the Reb M about the distance between the center of the lower VFD2 and the center of VFD3 (Kim et al., 2017).

Compared with sucrose, the RS of NHDC is 350–2,000 times stronger (Horowitz and Gentili, 1963; Kinghorn et al., 2010; Priya et al., 2011; Surana et al., 2006; Tiefenbacher, 2017; Yebra-Biurrun, 2013). The relative potency of NHDC was 1,837.46 which is within its MRS

Table 1 Relative potency of sweeteners to sucrose in affinity assay and relative sweetness to sucrose in human sensory evaluation

Sweeteners	EC_{50}	Relative potency[a] in affinity assay relative to sucrose (A)	MRS values[b] (B)	RS	References[c]	Log (A)	Log (B)
Sucrose	26 mM	1.00	1.00	1.00		0.00	0.00
Allulose	68.65 mM	0.38	0.37	0.70	Chung et al. (2012) Tiefenbacher (2017)	− 0.42	− 0.43
D-fructose	41.22 mM	0.63	0.62 ~ 0.95	1.17 ~ 1.80	Colonna et al. (2000) Featherstone (2015) Parker et al. (2010) Tiefenbacher (2017)	− 0.20	− 0.11
Saccharin	171.6 uM	151.52	107.03 ~ 374.61	200 ~ 700	Colonna et al. (2000) Featherstone (2015) Parker et al. (2010) Tiefenbacher (2017) Yebra-Biurrun (2013)	2.18	2.38
Rebaudioside A	19.43 uM	1,338.14	282.5 ~ 847.51	100 ~ 300	Carakostas et al. (2008) Goyal et al. (2010) Kemp and Lindley (2009)	3.13	2.75
Rebaudioside M	29.54 uM	880.16	754.32 ~ 1,320.05	200 ~ 350	Parkash et al. (2014)	2.94	3.02
NHDC	14.15 uM	1,837.46	626.37 ~ 3,579.24	350 ~ 2,000	Horowitz and Gentili (1963) Kinghorn et al. (2010) Priya et al. (2011) Surana et al. (2006) Tiefenbacher (2017) Yebra-Biurrun (2013)	3.26	3.32

MRS, molar relative sweetness; RS, relative sweetness

[a]Relative potency = EC50 in affinity assay (sucrose)/EC50 in affinity assay (compound)

[b]MRS value = RS value (from the literature) x [molecular weight (compound)/molecular weight (sucrose)]

[c]Reference to RS of sweeteners in human sensory evaluation

range (626.37–3,579.24). These data indicate that the relative potency in the affinity assay corresponds more with the MRS than RS. Thus, the MRS, which accounts for the binding affinity of the ligand at a molecular level, seems to reflect the efficacy of relative potency measured by the in vitro affinity assay. Through the in vitro affinity assay, the EC_{50} of sweeteners was similar to the sweetness property of bulk and high-intensity sweeteners, and relative potency of the sweeteners was confirmed as similar or within the ranges of the human sensory evaluation obtained from previous literature. To determine the correlation between the relative potency by the affinity assay and the mean MRS values, correlation analysis was performed by Pearson's correlation analysis. The log numbers of the calculated relative potency of sweeteners by the affinity assay showed a strong and positive correlation ($r = 0.9943$) with the mean MRS value (Fig. 3).

Previous research confirmed the correlation between the sensory evaluation and not only in vitro assay for isovanillic sweeteners (Bassoli et al., 2008) and high intensity sweeteners (Li and Servant, 2008), but also human tongue-like nanovesicle-based bioelectronic tongues (Song et al., 2014). In the present study, data correlation was shown between the in vitro activity assay and human sensory evaluation for high-intensity sweeteners as well as for bulk sweeteners. The relative potency of sweetness in vitro affinity assay was correlated well with the MRS by human sensory evaluation regardless of the intensities of sweetness.

This study identified the relative potency using EC_{50} for bulk and high-intensity sweeteners, which were consistent with the degree of sweetness of various sweeteners. The relative potency of the sweeteners to sucrose by the in vitro affinity assay displayed a good correlation with the calculated MRS from the previous human sensory evaluation studies. Currently, the determination of sweetener quality relies heavily on human sensory tests. However, it is difficult to explain the bitterness of aftertaste of high concentrations of sweeteners and the enhancement of sweetness with mixed sweeteners in human sensory tests. It

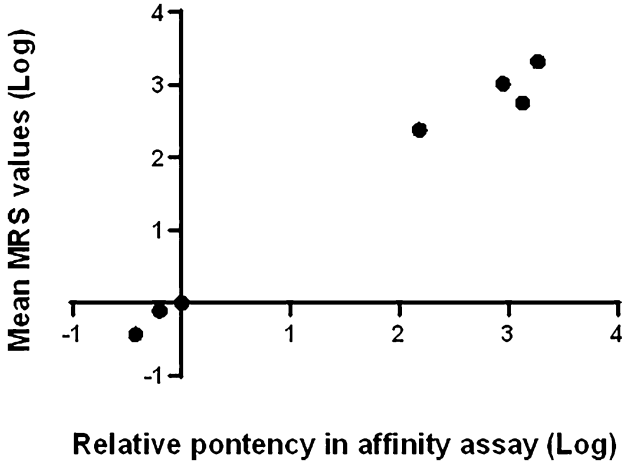

Fig. 3 Correlation analysis between the potency of sweeteners relative to sucrose by in vitro affinity assay and the mean MRS values. Pearson correlation analysis was performed to examine the relationship between the mean MRS values and the log numbers of calculated potency of sweeteners (sucrose, D-fructose, allulose, saccharin, Reb A, Reb M, and NHDC) relative to sucrose by the human sweet taste receptor activity assay. Statistical significance was set at $P < 0.05$. MRS, molar relative sweetness; NHDC, neohesperidin dihydrochalcone; Reb, rebaudioside

remains to be determined whether the mechanism of taste receptor-ligand binding activity can be analyzed for off-taste in mixed sweeteners by an in vitro activity assay with bitter receptors.

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Declarations

Conflict of interest The authors have no conflict of interest to declare.

References

Adler E, Hoon MA, Mueller KL, Chandrashekar J, Ryba NJ, Zuker CS. A novel family of mammalian taste receptors. Cell. 100: 693-702 (2000)

Ahn SR, An JH, Song HS, Park JW, Lee SH, Kim JH, Jang J, Park TH. Duplex bioelectronic tongue for sensing umami and sweet tastes based on human taste receptor nanovesicles. ACS Nano. 10: 7287-7296 (2016)

Baghirova S, Hughes BG, Hendzel MJ, Schulz R. Sequential fractionation and isolation of subcellular proteins from tissue or cultured cells. MethodsX. 2: 440-445 (2015)

Bassoli A, Laureati M, Borgonovo G, Morini G, Servant G, Pagliarini, E. Isovanillic sweeteners: sensory evaluation and in vitro assays with human sweet taste receptor. Chemosensory Perception. 1: 174-183 (2008)

Behrens M, Blank K, Meyerhof W. Blends of non-caloric sweeteners saccharin and cyclamate show reduced off-taste due to TAS2R bitter receptor inhibition. Cell Chemical Biology. 24: 1199-1204 (2017)

Carakostas MC, Curry LL, Boileau AC, Brusick DJ. Overview: the history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. Food and Chemical Toxicology. 46: S1-S10 (2008)

Chandrashekar J, Hoon MA, Ryba NJ, Zuker CS. The receptors and cells for mammalian taste. Nature. 444: 288-294 (2006)

Chaudhari N, Roper SD. Review series: the cell biology of taste. Journal of Cell Biology. 190: 285-296 (2010)

Chung MY, Oh DK, Lee KW. Hypoglycemic health benefits of D-psicose. Journal of Agricultural and Food Chemistry. 60: 863-869 (2012)

Colonna WJ. Samaraweera U, Clarke MA, Cleary M, Godshall MA, White JS. Sugar. Vol 19. pp. 1–67. In: Kirk-Othmer encyclopedia of chemical technology. Wiley, New York, NY, USA (2000)

DuBois GE. Molecular mechanism of sweetness sensation. Physiology and Behavior. 164: 453-463 (2016)

Featherstone S. Ingredients used in the preparation of canned foods. 4th ed. Woodhead, UK. pp. 147-211 (2015)

Fernstrom JD, Munger SD, Sclafani A, de Araujo IE, Roberts A, Molinary S. Mechanisms for sweetness. Journal of Nutrition. 142: 1134S-1141S (2012)

Goyal SK, Samsher, Goyal RK. Stevia (Stevia rebaudiana) a biosweetener: a review. International Journal of Food Sciences and Nutrition. 61(1): 1-10 (2010)

Hellfritsch C, Brockhoff A, Stähler F, Meyerhof W, Hofmann T. Human psychometric and taste receptor responses to steviol glycosides. Journal of Agricultural and Food Chemistry. 60: 6782-6793 (2012)

Horowitz RM. Gentili B. Dihydrochalcone derivatives and their use as sweetening agents. U.S. Patent 3,087,821 (1963)

Kemp SE, Lindley MG. Developments in sweeteners for functional and speciality beverages. Woodhead, UK. pp. 39-54 (2009)

Kim SK, Chen Y, Abrol R, Goddard WA, Guthrie B. Activation mechanism of the G protein-coupled sweet receptor heterodimer with sweeteners and allosteric agonists. Proceedings of the National Academy of Sciences. 114: 2568-2573 (2017)

Kinghorn AD, Chin YW, Pan L. Jia Z. Natural products as sweeteners and sweetness modifiers. Vol 3. pp. 269–315 In: Comprehensive Natural Products II: Chemistry and Biology. Mander L, Liu HW (Ed). Elsevier., Oxford, UK (2010)

Lagerström MC, Schiöth HB. Structural diversity of G protein-coupled receptors and significance for drug discovery. Nature Reviews Drug Discovery. 7: 339-357 (2008)

Li J, Ning Y, Hedley W, Saunders B, Chen Y, Tindill N, Hannay T, Subramaniam S. The molecule pages database. Nature. 420: 716-717 (2002a)

Li X, Servant G. Functional characterization of the human sweet taste receptor: high-throughput screening assay development and structural function relation. ACS Symposium Series. 979: 368-385 (2008)

Li X, Staszewski L, Xu H, Durick K, Zoller M, Adler E. Human receptors for sweet and umami taste. Proceedings of the National Academy of Sciences. 99: 4692-4696 (2002b)

Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS. Mammalian sweet taste receptors. Cell. 106: 381-390 (2001)

Njoman MF, Nugroho G, Chandra SDP, Permana Y, Suhadi S, Mujiono M, Hermawan AD Sugiono S. The vulnerability of human sensory evaluation and the promising senses instrumentation. British Food Journal. 119: 2145-2160 (2017)

Parker K, Salas M, Nwosu VC. High fructose corn syrup: production, uses and public health concerns. Biotechnology and Molecular Biology Review. 5: 71-78 (2010)

Prakash I, Markosyan A, Bunders C. Development of next generation stevia sweetener: Rebaudioside M. Foods. 3: 162-175 (2014)

Priya K, Gupta VRM, Srikanth K. Natural sweeteners: a complete review. Journal of Pharmacy Research. 4: 2034-2039 (2011)

Song HS, Jin HJ, Ahn SR, Kim D, Lee SH, Kim UK, Simons CT, Hong S, Park TH. Bioelectronic tongue using heterodimeric human taste receptor for the discrimination of sweeteners with human-like performance. ACS Nano. 8: 9781-9789 (2014)

Spakman D. How to broaden the applicability of the high potency sweeteners steviol glycosides: from enzymatic glycosylation to recombinant production. Groningen Biomolecular Sciences and Biotechnology Institute, Groningen, Netherlands pp. 1-21 (2015)

Surana SJ, Gokhale SB, Rajmane RA, Jadhav RB. Non-saccharide natural intense sweeteners–an overview of current status. Natural Product Radiance. 54: 270-278 (2006)

Tiefenbacher KF. The Technology of Wafers and Waffles I: Operational Aspects. Academic Press, Oxford, UK pp.123-225 (2017)

Xu H, Staszewski L, Tang H, Adler E, Zoller M, Li X. Different functional roles of T1R subunits in the heteromeric taste receptors. Proceedings of the National Academy of Sciences. 101: 14258-14263 (2004)

Yebra-Biurrun MC. Food and Nutritional Analysis | Sweeteners. Vol 3. pp. 471–481. In: Encyclopedia of Analytical Science. Worsfold P, Townshend A, Poole C, Miró M (Eds). Elsevier., Oxford, UK (2013)

Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJP, Zuker CS. The receptors for mammalian sweet and umami taste. Cell. 115: 255-266 (2003)

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