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The human bitter taste receptor, hTAS2R16, discriminates slight differences in the configuration of disaccharides

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ABSTRACT

Sweetness and bitterness are key determinants of food acceptance and rejection, respectively. Sugars, such as sucrose and fructose, are generally recognized as sweet. However, not all sugars are sweet, and even anomers may have quite different tastes. For example, gentiobiose is bitter, whereas its anomer, isomaltose, is sweet. Despite this unique sensory character, the molecular basis of the bitterness of gentiobiose remains to be clarified. In this study, we used calcium imaging analysis of human embryonic kidney 293T cells that heterologously expressed human taste receptors to demonstrate that gentiobiose activated hTAS2R16, a bitter taste receptor, but not hT1R2/hT1R3, a sweet taste receptor. In contrast, isomaltose activated hT1R2/hT1R3. As a result, these anomers elicit different taste sensations. Mutational analysis of hTAS2R16 also indicated that gentiobiose and β-D-glucopyranosides, such as salicin share a common binding site of hTAS2R16.

1. Introduction

Humans can discriminate the five basic tastes, saltiness, sourness, sweetness, bitterness, and umami [1]. In particular, sweetness and bitterness are important tastes because they are related to food acceptance and rejection, respectively [2]. Sweet taste qualities are generally regarded as appetitive sensations for energy sources, whereas bitter tastes prevent the ingestion of toxic substances.

Sucrose and fructose are recognized as sweet but not all sugars are sweet, and even anomers may have quite different tastes. For example, gentiobiose (6-*O*-β-D-glucopyranosyl glucose), which is contained in honey and wine [3–6], is bitter, whereas its anomer isomaltose (6-*O*-α-D-glucopyranosyl glucose) is sweet [7,8] (Fig. 1A).

Recent progresses in the molecular biology of taste have revealed that taste cells express receptors that recognize specific ligands and detect each of the basic tastes [9]. In mammals, two members of the T1R family of G protein-coupled receptors (GPCRs), T1R2 and T1R3, mediate the sweet taste sensation, and small sweet molecules, including sugars, are assumed to interact with Venus flytrap (VFT) domain of T1R2 [10–13]. On the other hand, response to bitterness is mediated by the hTAS2R family, which are GPCRs comprised of 25 members. Experiments with heterologous by expressed taste receptors have identified many of their cognate bitter ligands [14–20]. However, it remains unclear whether the bitterness of gentiobiose is elicited by hTAS2R activation.

We hypothesized that hTAS2R receptors mediate the bitterness of gentiobiose. To test this hypothesis, we used calcium imaging analysis of human embryonic kidney 293T (HEK293T) cells that heterologously expressed these receptors to examine their response to gentiobiose. Our results suggested that hTAS2R16, a human bitter taste receptor, is responsible for the bitterness of gentiobiose. Furthermore, since gentiobiose is a β-glucopyranoside that is structurally similar to salicin, we hypothesized that gentiobiose and salicin share a common binding site of hTAS2R16. Our mutational analysis of hTAS2R16 confirmed this hypothesis.

2. Materials and methods

2.1. Chemicals

Gentiobiose, D-(+)-trehalose dihydrate, D-(+)-cellobiose, lactulose, and palatinose were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). D-(+)-maltose monohydrate, isomaltose, D-(+)-lactose monohydrate, D-(+)-sucrose, D-(+)-melibiose monohydrate, galactinol dihydrate, and D-(+)-turanose were obtained from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan).

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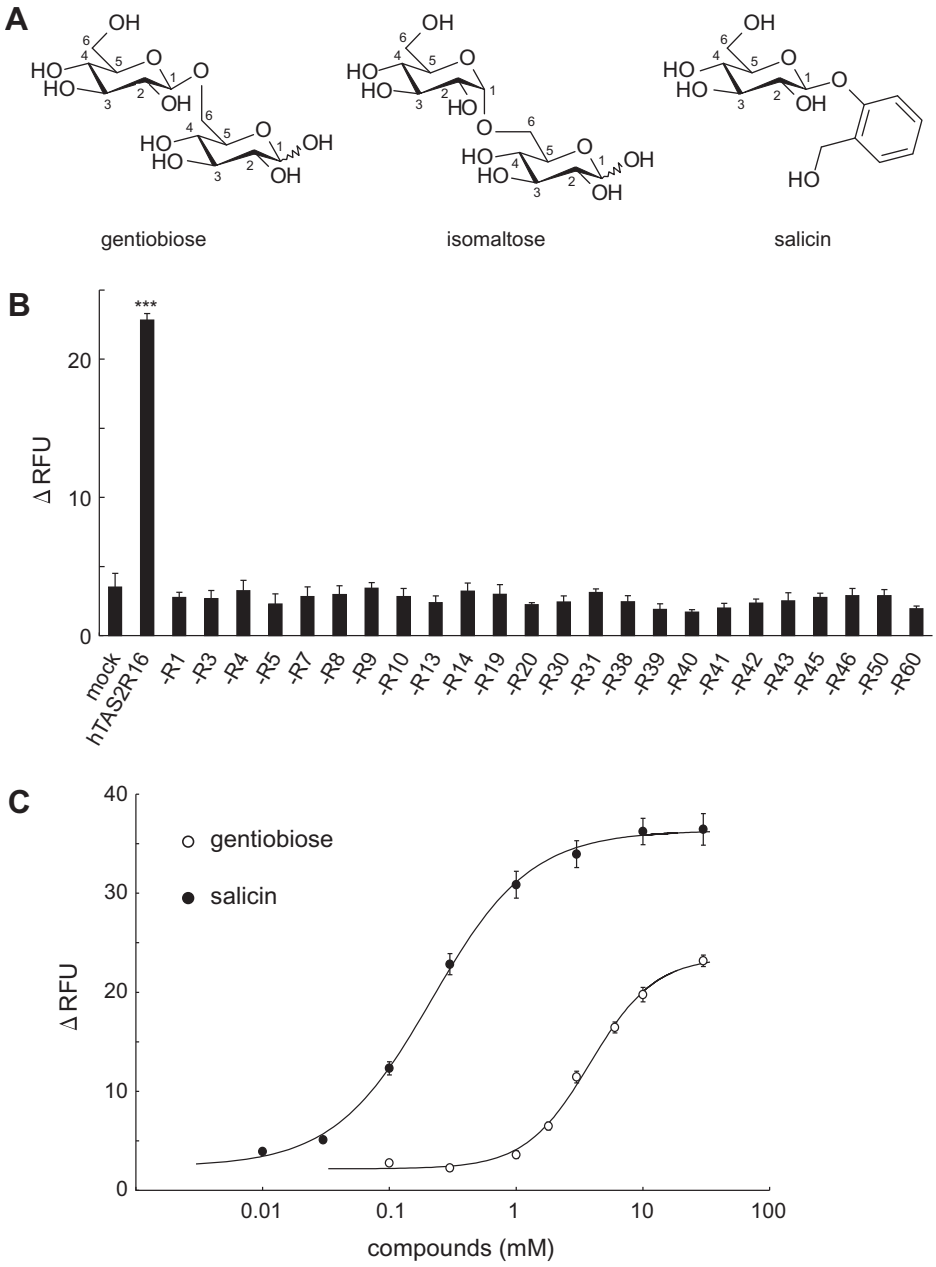

Fig. 1. Responses of human embryonic kidney cells (HEK293T) transiently expressing bitter taste receptors to gentiobiose. (A) The chemical structures of gentiobiose, isomaltose, and salicin. Numbers denote the positions of carbon atoms. (B) Responses of HEK293T cells coexpressing Gα16gust44 and each of 25 different human bitter taste receptors to 30 mM gentiobiose in cell-based assays. Recently, the names of several receptors have been changed: hTAS2R19 (hTAS2R48), hTAS2R20 (hTAS2R49), hTAS2R30 (hTAS2R47), and hTAS2R31 (hTAS2R44) (the former names are shown in parentheses). Each bar shows the mean and SEM from at least three independent experiments. The statistical significance of the differences between the response of mock-transfected cells and the cells that expressed human bitter taste receptors were determined by using one-way analysis of variance (ANOVA) followed by Dunnett's test. $^{***}p < 0.001$ vs. mock-transfected cells. (C) Dose-dependent responses of these cells to gentiobiose (open circles) and salicin (closed circles) in cell-based assays. Each data point shows the mean and SEM from at least three independent experiments. ΔRFU, change in relative fluorescence units.

Nigerose was purchased from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). The chemical structures of these disaccharides are shown in Fig. S1. Salicin was obtained from Nacalai Tesque, Inc. (Kyoto, Japan), and Glu–Glu (L-glutamyl-L-glutamic acid) from Peptide Institute, Inc. (Osaka, Japan).

2.2. Construction of expression plasmids for hTAS2Rs and the chimeric G protein

The genes encoding 25 hTAS2R proteins were tagged at the amino terminus with the sequence of the first 45 amino acids of rat somatostatin receptor type 3 (ssr3) [21], and then subcloned into the *Eco*R I-*Not* I or *Asc* I-*Not* I site of the pEAK10 expression vector (Edge Biosystems, Gaithersburg, MD). The expression plasmid for the chimeric G protein α subunit Gα16gust44 was subcloned into the pcDNA3.1 expression vector (Invitrogen, San Diego, CA, USA) [22]. Human TAS2R16 mutants were produced as described previously [23]. Briefly, mutations were introduced into ssr3-hTAS2R16 cDNA using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA), and then *Eco*R I-*Not* I fragments containing the desired mutation were ligated into the *Eco*R I-*Not* I site of the pEAK10 expression vector.