Journal of Oral Biosciences ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Journal of Oral Biosciences

journal homepage: www.elsevier.com/locate/job

Review

Binding properties between human sweet receptor and sweet-inhibitor, gymnemic acids

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ARTICLE INFO

Article history:
Received 17 February 2017
Received in revised form
13 April 2017
Accepted 8 May 2017

Keywords:
Sweet taste
TAS1R2+TAS1R3
Gymnemic acids
G protein-coupled receptors

ABSTRACT

Background: Gymnemic acids, triterpene glycosides, are known to act as human-specific sweet inhibitors. The long-lasting effect of gymnemic acids is diminished by γ-cyclodextrin. Here, we focus on the molecular mechanisms underlying the interaction between gymnemic acids and sweet taste receptor and/or γ-cyclodextrin by a sweet taste receptor assay in transiently transfected HEK293 cells.
Highlight: Application of gymnemic acids inhibited intracellular calcium responses to sweet compounds in HEK293 cells expressing human TAS1R2+TAS1R3 but not in those expressing the mouse sweet receptor Tas1r2+Tas1r3 after application of gymnemic acids. The effect of gymnemic acids was reduced after rinsing cells with γ-cyclodextrin. Based on species-specific sensitivities to gymnemic acids, we showed that the transmembrane domain of hTAS1R3 is involved in the sensitivity to gymnemic acids. Point mutation analysis in the transmembrane domain of hTAS1R3 revealed that gymnemic acids shared the same binding pocket with another sweet inhibitor, lactisole. Sensitivity to sweet compounds was also reduced by mixtures of glucuronic acid, a common gymnemic acid. In our molecular models, gymnemic acids interacted with a binding site formed in the transmembrane domain of hTAS1R3.
Conclusion: Gymnemic acids inhibit sweet responses in humans through an interaction between the glucuronosyl group of gymnemic acids and the transmembrane domain of hTAS1R3. Our molecular model provides a foundation for the development of taste modifiers.

Contents

1. Introduction

Humans perceive sweet taste to detect carbohydrates, which are a source of calories. The sweet taste signal conveyed to the brain is thought to play an important role in energy homeostasis. The TAS1R2+TAS1R3 heterodimer may be the sole receptor detecting sweet taste signals in taste bud cells embedded in the oral cavity (Fig. 1A). Previous studies using HEK293 cells demonstrated that the TAS1R2+TAS1R3 receptor can broadly respond to a variety of sweet chemicals including not only sugars, but also amino acids, peptides, proteins, and even artificial sweeteners that can bind the receptor molecules at different sites. Human psychophysical studies have shown that two chemical compounds, gymnemic acids (GAs) and lactisole, can act as sweet taste

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http://dx.doi.org/10.1016/j.job.2017.05.004
1349-0079/

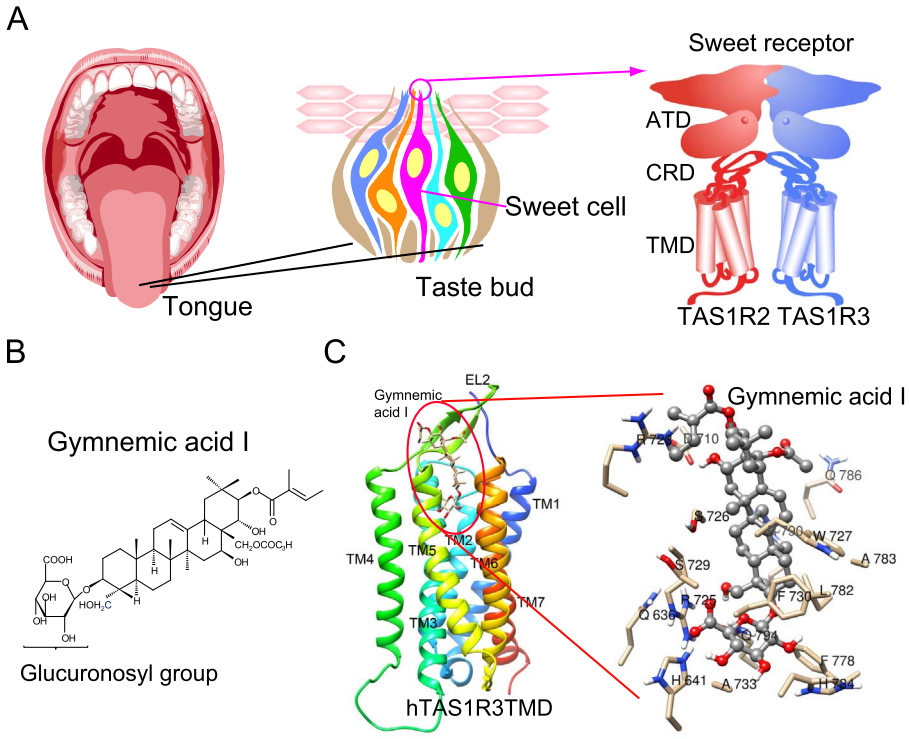

Fig. 1. Sweet-suppressing effect of gymnemic acids. (A) The sweet taste receptors are expressed in sweet receptor cells in taste buds. TAS1R2+TAS1R3 is composed of an amino-terminal domain (ATD), cysteine-rich domain (CRD), and transmembrane domain (TMD). (B) Molecular structure of gymnemic acid I. (C) Molecular model showing gymnemic acid I docked to the TMD of hTAS1R3. An overall view (left) and detailed view (right) are shown. Gymnemic acid I is shown in a ball and stick representation. Models are colored by atom type. This figure is modified from Ref. [31].

inhibitors to inhibit perceived sweetness, including for sweet chemicals with different chemical structures [1–4]. Therefore, the potential binding sites of the inhibitors on the sweet receptor and the mechanism by which these inhibitors block the activation of the receptor in response to a variety of sweet chemicals with different binding sites should be determined.

GAs, which are saponins of triterpene glycoside, are sweet inhibitors present in the leaves of the plant *Gymnema sylvestre*; this plant is native to central and western India (Fig. 1B). GAs are composed of several types of homologs and selectively suppress sweet taste responses for 30–60 min without affecting the responses to salty, sour, and bitter compounds [1–3]. The sweet-suppressing effect of GAs is specific to humans and chimpanzees, but does not occur in rodents and is diminished by the application of γ-cyclodextrin (CD) to the tongue [5–9]. GAs are also known to inhibit intestinal glucose absorption and reduce plasma glucose and insulin levels [10–12].

In this review, we focus on the molecular mechanisms underlying the interaction between the sweet receptor and GAs.

2. Sweet receptor

Sweet substances are detected by the TAS1R family belonging to class C G-protein coupled receptors, including the metabotropic glutamate receptors, calcium-sensing receptor, and other taste/olfactory receptors [13–21]. TAS1R2 and TAS1R3 form a heterodimeric complex to function as a sweet taste receptor. Based on sequence similarity, the structures of TAS1Rs consist of three major domains: a large extracellular amino-terminal domain (ATD), cysteine-rich linker domain (CRD), and heptahelical transmembrane domain (TMD) [21]. The ATD, also known as the Venus flytrap module, consists of lobes 1 and 2 and can be in an “open” or “closed” conformation to bind with ligands. The ATD is connected to the TMD by the CRD (Fig. 1A).

Recent studies have shown that TAS1R2+TAS1R3 have multiple binding sites for structurally diverse sweet substances and sweet-modifiers. Low-molecular-weight sweet compounds bind the interaction site of the Venus flytrap module formed by the bottom of lobe 1 and top of lobe 2 in TAS1R2 [22,23]. The ATD of human (h) TAS1R3 is important for sensitivity to the taste-modifying protein neoculin [24]. Sensitivity to brazzein is determined by the CRD of hTAS1R3 and the ATD of hTAS1R2 [25,26]. The TMD of hTAS1R3 is involved in the sensitivity to artificial sweetener cyclamate and neohesperidin dihydrochalcone and sweet inhibitors such as lactisole, phenoxy herbicides, and fibrates [4,27–29].

Miraculin, a glycoprotein, induces sweet taste in humans after acidification of the tongue. The magnitude of sweet responses induced by miraculin is higher following acidification caused by weak acids compared to that caused by strong acids [30]. Replacement of histidine with alanine indicated that protonation of hTAS1R2 affects the activation of the sweet receptor. We found that intracellular acidification is required for complete activation of the sweet receptor by miraculin [30].

3. Interaction site for GAs

We first examined whether GAs directly interact with the human sweet receptor by conducting a sweet receptor assay in HEK293 cells transiently expressing hTAS1R2, hTAS1R3, and Gα16-gust44 [31]. We monitored $[Ca^{2+}]_i$ responses to various sweet substances such as SC45647, saccharin, aspartame, cyclamate, D-tryptophan, and sucrose [31]. Calcium responses to these substances were reduced or completely absent after the application of GAs, indicating the sweet-suppressing effect of GAs. GAs did not affect the EC_{50} value of concentration-dependent responses to saccharin, but reduced the maximum responses. This suggests that

Please cite this article as: Sanematsu K, et al. Binding properties between human sweet receptor and sweet-inhibitor, gymnemic acids. J Oral Biosci (2017), http://dx.doi.org/10.1016/j.job.2017.05.004