

Sweet-taste-suppressing compounds: current knowledge and perspectives of application

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Abstract Sweet-tasting compounds are recognized by a heterodimeric receptor composed of the taste receptor, type 1, members 2 (T1R2) and 3 (T1R3) located in the mouth. This receptor is also expressed in the gut where it is involved in intestinal absorption, metabolic regulation, and glucose homeostasis. These metabolic functions make the sweet taste receptor a potential novel therapeutic target for the treatment of obesity and related metabolic dysfunctions such as diabetes. Existing sweet taste inhibitors or blockers that are still in development would constitute promising therapeutic agents. In this review, we will summarize the current knowledge of sweet taste inhibitors, including a sweet-taste-suppressing protein named gurmarin, which is only active on rodent sweet taste receptors but not on that of humans. In addition, their potential applications as therapeutic tools are discussed.

Keywords Gurmarin · Taste receptor · Inhibitor · Sweetener · GPCR · *Gymnema sylvestre*

Introduction

Nowadays, there is an expanding consumer interest in herbal medicines aimed at reducing caloric intake and treating diabetes due to the side effects associated with chemical sweeteners and conventional antidiabetic drugs (Dubois

and Prakash 2012). Several plants used by native people for a long time are now becoming recognized as alternative therapeutics for lowering high blood glucose levels and treating obesity. Sweetness is mediated by a large number of sweet-tasting substances, which are chemically diverse compounds, including natural sugars as well as noncarbohydrate synthetic and natural substances (Behrens et al. 2011; DuBois 2008). In the early 2000s, it was a major breakthrough when it was discovered that sweet taste is largely mediated by a single sweet taste receptor (Chandrashekar et al. 2006; Li et al. 2002; Nelson et al. 2001). This receptor is a heterodimer composed of two members of the taste receptor family 1, T1R2 and T1R3, which belong to the family of class C G protein-coupled receptors (GPCRs). Members of class C GPCRs are generally organized into three major domains. In addition to a transmembrane heptahelical domain (TM), this class of receptors shares a large N-terminal domain so-called venus flytrap (VFT) motif, which forms the primary ligand binding site (Pin et al. 2003), linked to the TM via cysteine-rich domain (CRD). Numerous studies conducted on T1R2/T1R3 taste receptor have revealed multiple functional binding sites for natural sugars, some noncaloric sweeteners, and sweet-tasting proteins (Assadi-Porter et al. 2010; Cui et al. 2006; Jiang et al. 2004; Koizumi et al. 2007; Liu et al. 2011; Masuda et al. 2012; Nie et al. 2005, 2006; Winnig et al. 2005, 2007; Xu et al. 2004). In addition, cell-based assays for taste receptors have been used successfully for the discovery of sweet taste enhancers (Servant et al. 2010; Zhang et al. 2010). However, sweet-taste-suppressing compounds have received little attention. Sweetness inhibitors allow food developers to take advantage of the functional properties of sugar (e.g., texture) without producing an overly sweet product. For example, lactose-free milk is perceived as a little sweeter than regular skim milk because lactose is enzymatically broken down into simple sugars (Ohmiya et al. 1977). To overcome this problem, a sweetness inhibitor can be added to lower the sweetness of lactose-free milk.

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Recent data have shown that the T1R2/T1R3 receptor is also expressed in a number of non-taste tissues, including endocrine cells of the gastrointestinal tract, where it may be involved in luminal glucose sensing, the release of some satiety hormones, the expression of glucose transporters, and the maintenance of glucose homeostasis (Dyer et al. 2005; Margolskee et al. 2007; Rozengurt et al. 2006). This newly recognized role of the sweet taste receptor in glucose sensing has renewed interest in taste receptor modulation (Mace et al. 2009; Margolskee et al. 2007). Indeed, this novel receptor function suggests that antagonist compounds can constitute a new therapeutic pathway to treat obesity and certain related metabolic dysfunctions including diabetes. This review will focus on the current state of sweet-taste-suppressing compounds, particularly gurmarin, which is only active on rodent T1R2/T1R3 but not on the human homolog. Its application for sweet taste mechanism studies is analyzed.

Sweetness inhibitors

Synthetic sweetness inhibitors

The number of sweetness inhibitors known hitherto is very limited in comparison to sweet-tasting compounds (Kurihara 1992). Synthetic sweetness inhibitors include amiloride (Imada et al. 2010), *N*-(4-cyanophenyl)-*N'*-[(sodiosulfo)methyl]urea (Muller et al. 1992) in humans, methyl 4,6-dichloro-4,6-dideoxy- α -D-galactopyranoside, *p*-nitrophenyl α -D-glucopyranoside, and chloramphenicol in gerbils (Jakimovich 1983; Vlahopoulos and Jakimovich 1986a, b), and alloxan (Zawalich 1973) and iodoacetic acid (Noma and Hiji 1972) in rats (Fig. 1). In mice, zinc chloride and copper chloride have been reported to inhibit chorda tympani nerve responses to sweet without affecting responses of the other taste stimuli (Iwasaki and Sato 1984, 1986). In addition, zinc sulfate (Keast 2003; Keast et al. 2004) and copper chloride (Somenarain and Jakimovich 1990) have also been reported to inhibit sweetness perceived by humans and gerbils, respectively. These inhibitory compounds are suspected to act on the sweet taste receptor, but to date, the sites of action of these inhibitors have not been identified.

Plant-derived triterpenoid sweetness inhibitors

A number of plant-derived triterpenoids have been reported to reduce the sweet taste recognition in humans, including hodulcin from *Hovenia dulcis* (Kennedy et al. 1988), gymnemic acid from *Gymnema sylvestre* R. Br. (Liu et al. 1992), and ziziphin from *Ziziphus jujuba* (Meiselman et al. 1976) (Fig. 2). Gymnemic acid, a triterpenoid saponin, is the most studied of the plant-derived inhibitory compounds, consisting

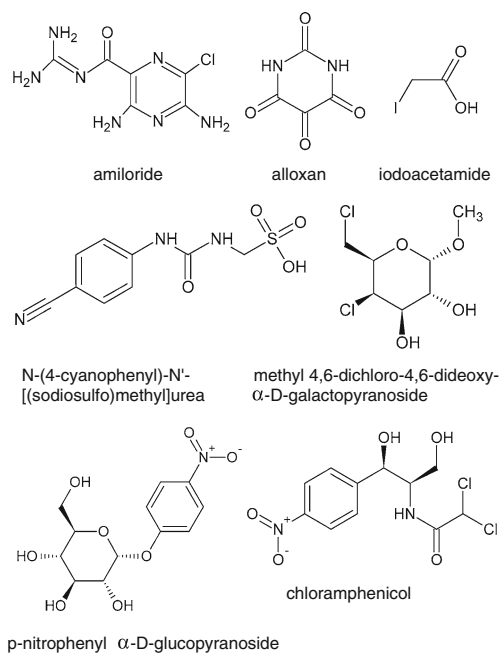


Fig. 1 Chemical structures of synthetic anti-sweet compounds. Structures were generated by the ChemSketch Freeware 10.00

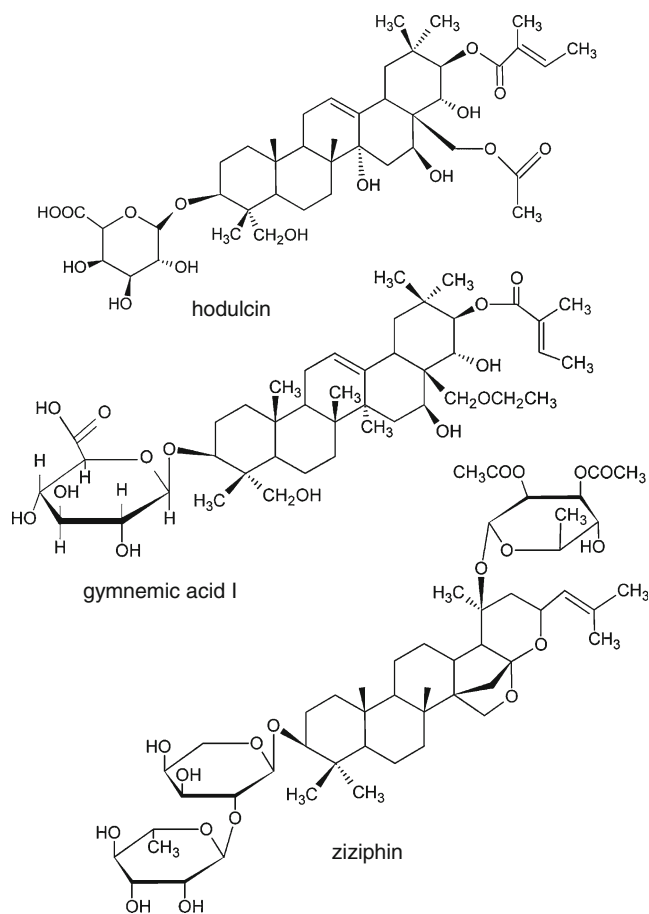


Fig. 2 Chemical structures of natural anti-sweet compounds

of a mixture of related acidic substances. Interestingly, physiological studies have revealed that sweet taste inhibition by gymnemic acid was species-specific (Diamant et al. 1965). For instance, no significant effect was observed in hamsters, rats (Hellekant and Gopal 1976), rabbits, and pigs, although in old world monkeys and humans, the sweet taste perception was greatly affected (Hellekant et al. 1985). Gymnemic acid suppresses the sweet taste of numerous compounds in chimpanzees, including sucrose, saccharin, acesulfame K, aspartame, cyclamate, amino acids such as glycine, D-tryptophan, D, L-alanine, and D-leucine, as well as two sweet-tasting proteins, thaumatin and monellin (Hellekant et al. 1996, 1998). To date, the binding sites of these plant-derived sweetness inhibitors have not been characterized.

The most studied low molecular weight sweetness inhibitor is lactisole (Fig. 3). Lactisole is the sodium salt of 2-(4-methoxyphenyl)propionic acid, which was isolated from coffee beans (Schiffman et al. 1999). It is commercialized under the trade name Cypha™ as an artificial flavor enhancer. Lactisole inhibits sweet and umami taste perception in humans but is ineffective in rats and mice (Galindo-Cuspinera and Breslin 2006; Sclafani and Perez 1997). Similar to gymnemic acid, lactisole is a broad-acting

inhibitor of all or most sweeteners tested in humans (Schiffman et al. 1999). More recently, various phenoxyherbicides and lipid-lowering fibrates with chemical similarity to lactisole (Fig. 3) were demonstrated to inactivate the human sweet taste receptor at micromolar concentrations but not the corresponding rodent receptors (Maillet et al. 2009). Since these compounds are used in agriculture and medicine, much more research needs to be done on the effects of these molecules in human and animal physiology. Surprisingly, some artificial sweeteners such as saccharin and acesulfame K inhibit sweet taste at high concentration, contributing to the off-taste properties of these sweeteners (Galindo-Cuspinera et al. 2006).

Sweet-taste-suppressing proteins

To date, only two sweet-taste-suppressing proteins have been identified, i.e., gurmarin and riboflavin-binding protein (RBP). RBP is found in chicken egg white at approximately 9 mg/kg (Rhodes et al. 1959). RBP is made of 219 amino acid residues with nine disulfide bonds (Hamazume et al. 1987). RBP has been observed to bind riboflavin tightly, a type of B vitamin, in a 1:1 molar ratio. The family of hen RBP is composed of three proteins from different organs: egg white, egg yolk, and plasma. These proteins are encoded by the same gene but undergo different posttranslational modifications, such as glycosylation or proteolytic cleavage. Both egg yolk and egg white RBPs have been reported to selectively inhibit the taste of sweet proteins, but not carbohydrate or artificial sweeteners, albeit with low inhibition potency (Maehashi et al. 2007). Egg white RBP was reported to exhibit higher sweet-suppressing activity than egg yolk RBP, although the mechanism for RBP sweetness inhibition is not known (Maehashi et al. 2007). RBPs seem not to have proteolytic activity or direct interaction with the sweet-tasting proteins. However, an interaction between RBP and sweet taste receptor has been proposed (Maehashi et al. 2007). Interestingly, bitter-suppressing properties of RBP have also been reported, while it has no effect on salty, sour, and umami tastes (Maehashi et al. 2007, 2010).

Gurmarin discovery: a brief historical overview

In the course of their study on the physiological effects of *G. sylvestre*, Imoto et al. (Imoto et al. 1991) reported that hot water extracts of leaves reduced the neural responses to sweet stimuli in rats, but not in humans. In contrast, previous studies had shown that gymnemic acid reduced sweet responses in humans, but not in rodents. These facts suggested strongly that *G. sylvestre* contained hitherto unknown taste-suppressive substances. Accordingly, subsequent purification of the active principle did not identify another

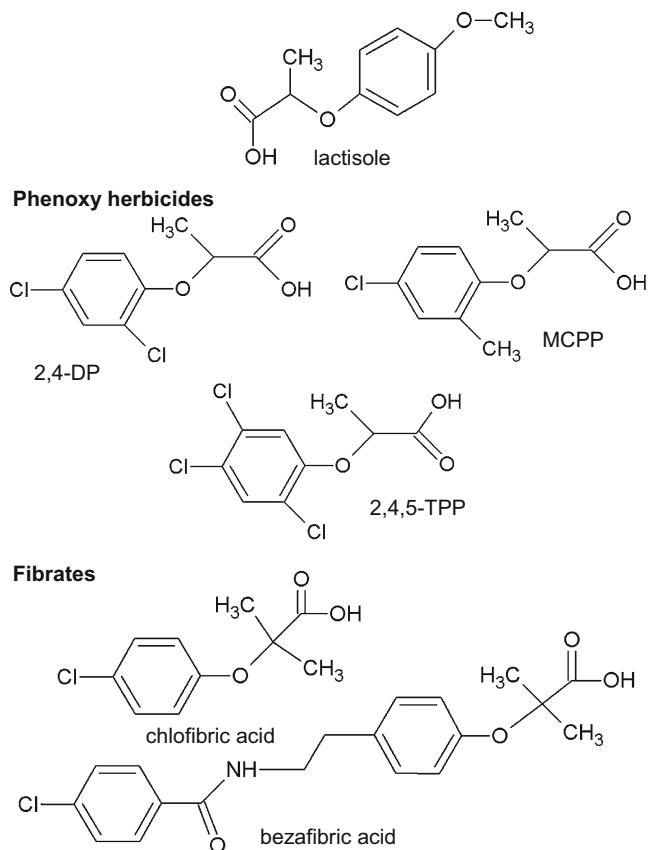


Fig. 3 Chemical structures of lactisole and derivative structures. Structural similarities between lactisole, several phenoxy herbicides, and fibrates are shown

triterpenoid saponin but led to the discovery of the first sweetness inhibitor protein. The authors proposed the name “gurmarin” following the folkloric Hindi name “Gurmar” meaning “sugar destroyer”.

Biochemical properties

Gurmarin consists of 35 amino acids with three disulfide bridges and an N-terminus, which is blocked by pyroglutamic acid (Fig. 4a). The isoelectric point of natural gurmarin was determined to be 4.5 using isoelectric focusing experiments (Imoto et al. 1991). The inhibitory effect of gurmarin occurs at low concentrations with an IC_{50} value of 0.03 $\mu\text{g/ml}$ or 7.1 nM (Imoto et al. 1991; Sigoillot et al. 2012). A peculiar feature of gurmarin is that although the inhibitory effect is reversible, several hours (>2–3 h) are necessary for a complete recovery of the sweet taste function in rats (Imoto et al. 1991). However, if the tongue was rinsed with β -cyclodextrin (β -CD) solution, rapid recovery of gurmarin-suppressed sweetener responses was observed in recordings of the peripheral chorda tympani nerve (Ninomiya et al. 1998) or in behavioral tests in mice (Murata et al. 2003). β -CD can form inclusion complexes with tyrosine residues and more weakly with tryptophan residues of gurmarin hydrophobic cluster (Imoto et al.

2001) with an affinity K_d value of 3.9 mM. It is likely that the formation of hydrophilic β -CD inclusion complexes facilitates the removal of gurmarin from the taste tissue. However, the precise molecular mechanisms of the shortened recovery rates remain unclear.

Isolation from natural source and chemical synthesis of gurmarin

The natural source of gurmarin is the leaves of *G. sylvestre*. Generally, the leaves are harvested after cutting and pulling down the main stem to the ground. Due to this destructive prevailing practice, the availability of *G. sylvestre* is decreasing day by day. Until now, the entire crop is harvested from wild plants, and the species is becoming vulnerable (Pandey and Yadav 2010). To circumvent the ecological problem of obtaining native gurmarin purified from *G. sylvestre*, the chemical synthesis of gurmarin using t-Boc-protected solid-phase peptide synthesis techniques has been established (Fletcher et al. 1999). Purification and folding of synthetic gurmarin led to functional protein with structural properties similar to that of the native gurmarin (Fletcher et al. 1999).

Heterologous expression of gurmarin

All of the functional studies of gurmarin have relied on either native protein extracted from the plant or chemically synthesized gurmarin. Recently, we reported the first recombinant expression of gurmarin using the methylotrophic yeast *Pichia pastoris* production system (Sigoillot et al. 2012). To express large quantities of gurmarin, we designed a synthetic gene-coding gurmarin and optimized it for expression in *P. pastoris*. We demonstrated that recombinant gurmarin with an N-terminal glutamic acid residue instead of a pyroglutamic acid residue improves the yield of production and also exhibits a sweet-taste-suppressing effect on heterologously expressed rat sweet taste receptor (Sigoillot et al. 2012). Since *P. pastoris* grows rapidly on inexpensive media, it is possible to quickly and cheaply produce and investigate large numbers of mutant proteins suitable for structure-function studies (Cregg et al. 2000). Furthermore, the *P. pastoris* system allowed production of ^{15}N -labeled gurmarin useful for future nuclear magnetic resonance (NMR) investigations (Sigoillot et al. 2012).

Sequence homologies and three-dimensional structure of gurmarin

The three-dimensional (3D) structure of the native or chemically synthesized gurmarin has been solved by NMR spectroscopy (Arai et al. 1995; Fletcher et al. 1999). Gurmarin adopts a compact structure containing an antiparallel β -sheet, several well-defined β -turns, and a knottin fold motif (Fig. 4b, c) with an *abcabc* disulfide topology, which means that the

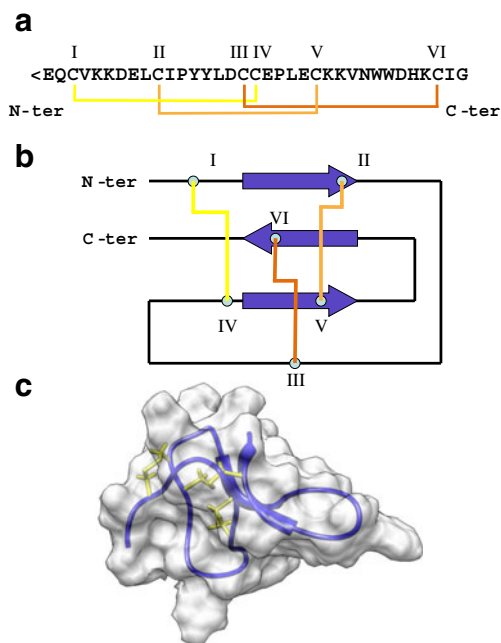


Fig. 4 Structural properties of gurmarin. **a** Amino acid sequence of gurmarin, highlighting the disulfide bridges, pyroglutamic acid: <E. **b** Schematic representation of the inhibitor cystine knot. The β -strands are drawn as blue arrows, the cysteine residues are labeled I–VI from the N- to C-terminus. Disulfide bridges are represented as yellow to orange lines. **c** 3D structure of gurmarin. The backbone of gurmarin is represented in blue cartoon; the β -strands are shown as arrows with disulfide bridges colored in yellow. The figure has been prepared with Pymol 1.3 using gurmarin NMR structures (PDB code: 1C4E)

first cysteine residue in the protein sequence forms a disulfide bridge with the fourth and so on. Gurmarin also has a consecutive Cys-Cys sequence and is stable against selective cleavage by proteases, even under conditions involving high temperature, low pH, and the presence of urea (Imoto et al. 1991). Moreover, the yield and the activity of gurmarin were largely unchanged even after an hour of high-temperature (90 °C) extraction from *G. sylvestre* leaves. It has also been reported that aqueous solution of gurmarin can be stored for more than 2 months without loss of activity (Imoto et al. 1991). Interestingly, the gurmarin crystal structure revealed that two tyrosine residues and two tryptophan residues, whose side chains are all directed outwardly, form a hydrophobic cluster together with the amino acid side chains of Leu 9, Ile 11, and Pro 12 (Fig. 5). It has been suggested that this hydrophobic patch can act as the site for interaction with the sweet taste receptor (Arai et al. 1995).

A search in protein databases yielded no sequences with significant homology to gurmarin. The structural comparison of the knottin proteins (Carugo et al. 2001) revealed that gurmarin belongs to the Toxin 7 superfamily. This family consists of several short spider neurotoxin proteins including many from the Funnel-web spider. Interestingly, the 3D structure of gurmarin is very similar to δ -atracotoxin (δ -ACTX), a spider neurotoxin, and the ω -conotoxin, a neurotoxin produced by fish-eating marine snails of the *Conus* genus (Pallaghy et al. 1993). This similarity suggested that the sweet taste suppression capacity of gurmarin may result from modification of conductance properties of an ion channel involved in sweet taste transduction (Fletcher et al. 1997). However, despite this structural similarity, gurmarin activity does not mimic the effects of δ -ACTX (Fletcher et al. 1999).

Physiological characteristics of gurmarin: electrophysiological and behavioral studies

Gurmarin selectively inhibits the neural responses to sweet substances in rats (Imoto et al. 1991; Miyasaka and Imoto 1995) and mice (Ninomiya and Imoto 1995) without affecting responses to other basic taste stimuli, such as NaCl, HCl,

and quinine. Gurmarin also specifically attenuates sweet responses of gerbil taste cells (Uchida and Sato 1997). Moreover, lingual treatment with gurmarin diminishes physiological and behavioral responses to umami stimuli in mice (Nakashima et al. 2001; Ninomiya et al. 2000) and rats (Sako and Yamamoto 1999; Sako et al. 2003). In mice, it has been shown that the gurmarin inhibition is strain- and nerve-specific: only sweet responses transmitted via the chorda tympani, but not the glossopharyngeal nerve, were sensitive to gurmarin (Ninomiya and Imoto 1995; Ninomiya et al. 1997). Furthermore, it has been shown that rats fed with a diet containing *G. sylvestre* powder transiently decrease their sucrose preference (Katsukawa et al. 1999). The recovery of sucrose preference was correlated with the induction of salivary proteins, kallikreins 2 and 9 (Yamada et al. 2006). This has led the authors to suggest that these kallikreins may interact with aromatic amino acids of gurmarin to suppress gurmarin interaction with the taste system as a mechanism of defense against unfavorable reduction of sweet taste sensitivity. However, no experimental data have been provided to support this hypothesis.

Gurmarin targets the sweet taste receptor

As previously mentioned, it is well established that gurmarin blocks sweet taste after direct treatment of the tongue surface, while intravenous injection of gurmarin has no effect on taste perception (Miyasaka and Imoto 1995). Based on these observations, it was concluded that gurmarin mediated the suppression of sweetness by interacting with a sweet taste receptor protein located at the apical side of taste receptor cells, years ahead of molecular identification of taste receptors (Miyasaka and Imoto 1995). Recently, this assumption has been unequivocally verified by experiments with the recombinant sweet taste receptor, which demonstrated that pretreatment with gurmarin inhibited sweetener-evoked calcium mobilization in human embryonic kidney (HEK) 293 cells transiently expressing the mouse sweet taste receptor (Margolskee et al. 2007).

Structure and function of the sweet taste receptor

Despite their highly diverse chemical structures, most if not all sweet-tasting substances are detected by a universal sweet taste receptor (Behrens and Meyerhof 2011). Two members of the T1Rs, T1R2 and T1R3, form an obligate heterodimer to constitute a functional sweet taste receptor (Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001; Sainz et al. 2001). Similarly, the third known member of this family, T1R1 also heterodimerizes with T1R3 to constitute an umami taste receptor in humans or a taste receptor for L-amino acids in rodents (Li et al. 2002; Nelson et al. 2002). T1Rs are assigned to class C of the GPCR superfamily, thus sharing conserved

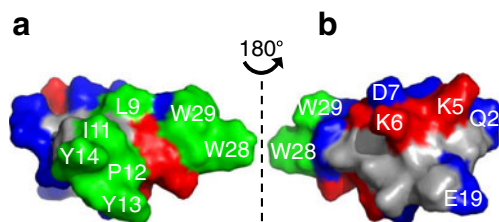


Fig. 5 Structure–activity relationship: 3D structure of gurmarin and putative binding site. **a** Surface representation of gurmarin illustrating the hydrophobic cluster (green); the acidic and basic amino acid residues are shown in blue and red, respectively. **b** Opposite face (rotation of 180°) of the hydrophobic cluster. The figure has been prepared with Pymol 1.3 software

structural features with, e.g., metabotropic glutamate receptors and the calcium-sensing receptor (Max et al. 2001; Montmayeur et al. 2001). The crystal structures of T1Rs are yet to be solved at atomic resolution, but sequence similarity and experimental evidence indicated that their protomers overall share the characteristic molecular structure of other class C GPCRs (Fig. 6a). In particular, the large VFT harbors the orthosteric ligand binding site, while the TM of class C GPCRs, including the sweet and umami taste receptors, form allosteric binding sites for various modulators (Kniazeff et al. 2011).

However, unlike other class C GPCRs, both subunits of the T1R heteromers contribute to ligand binding (Fig. 6b). Hence, the receptor complexes contain multiple distinct ligand interaction sites which provide the basis for the broad molecular receptive range of the sweet taste receptor (Cui et al. 2006). During the last decade, the binding sites for several sweet compounds have been identified by means of biomolecular and biophysical experiments (Fig. 6b). Sucrose and glucose appear to bind to the VFT of both T1R2 and T1R3 subunits (Maitrepierre et al. 2012; Nie et al. 2005). Other substances, such as aspartame, interact with the VFT of only one, the human T1R2 subunit (Liu et al. 2011; Masuda et al. 2012;

Xu et al. 2004). In contrast to most class C GPCRs, the allosteric binding site at the TM of human T1R3 interacts not only with modulators of the receptor function, such as lactisole, but also with the full receptor agonists neohesperidin dihydrochalcone (NHDC) and cyclamate (Winnig et al. 2005, 2007; Xu et al. 2004). The binding mode of the sweet-tasting proteins monellin and brazzein has not been resolved in detail yet; however, experimental results indicated a role of human T1R2 VFT and T1R3 CRD (Jiang et al. 2004). Many of these mapping studies took advantage of pronounced species differences in terms of sensitivity to various sweet substances, which can be accounted for by the sequence differences in the respective receptor subunits between rodents and humans (Galindo-Cuspinera et al. 2006; Jiang et al. 2004, 2005b; Koizumi et al. 2007; Li et al. 2011; Ohta et al. 2011; Winnig et al. 2005, 2007; Xu et al. 2004; Zhang et al. 2010). Moreover, the functional expression of interspecies hybrid and chimeric receptors permitted the investigation of binding sites of species-specific sweet-tasting compounds, such as aspartame, thaumatin, and NHDC (Ohta et al. 2011; Winnig et al. 2005, 2007; Xu et al. 2004).

Activation of the heterodimeric receptor complex T1R2/T1R3 by its sweet orthosteric agonists likely resembles that of the related homodimeric metabotropic glutamate receptor (mGluR). Elucidation of the crystal structure of the VFT of mGluR₁ in the absence and presence of the endogenous ligand glutamate revealed that the VFT changes its conformation in a dynamic equilibrium between an opened and a closed state (Kunishima et al. 2000). Agonist molecules seem to bind preferably to one lobe at the open conformation and stabilize the closed form by molecular interaction with the second lobe (for a detailed review, see Kniazeff et al. (2011)). Upon closure, the VFT of both protomers shift their relative orientation to each other, thus switching receptor conformation from resting to active state. This rearrangement of extracellular domains is transmitted to the TM via the CRD. In the case of the T1Rs, activation of the coupled G protein is probably mediated by the taste quality-specific subunit of the heterodimer, that is T1R2 in the sweet and T1R1 in the umami taste receptor (Sainz et al. 2007).

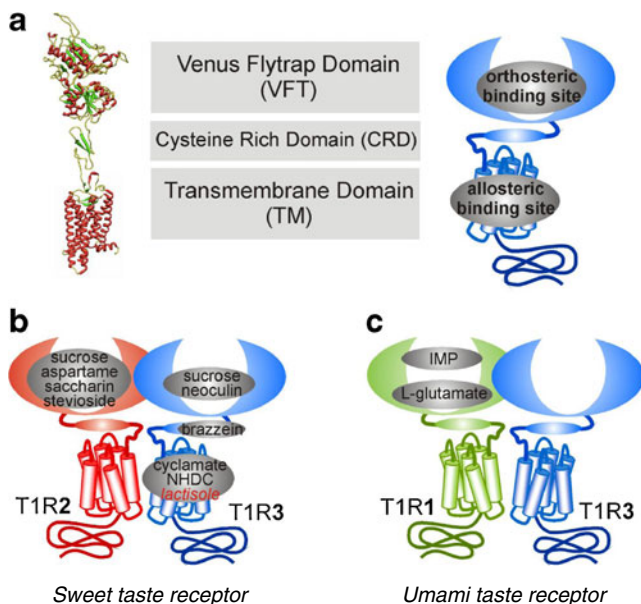


Fig. 6 Schematic of the structure and functional domains of the T1R receptors. **a** Ribbon representation and schema of human T1R3. The localization of functional domains and binding sites in the protomer are indicated according to the general configuration of class C GPCRs. **b** Examples of known ligand binding sites in the human sweet taste receptor heteromer. Aspartame, brazzein, cyclamate, neohesperidine dihydrochalcone, and lactisole are selective agonists of the human sweet taste receptor and do not activate rodent sweet taste receptors. **c** Examples of known ligand binding sites in the human umami taste receptor heteromer. 5'-ribonucleotides, such as IMP, enhance receptor responses to the umami compound L-glutamate by a cooperative binding mode

Mechanism of gurmarin action on the taste receptor

Gurmarin inhibits sweetener-mediated calcium responses of cells expressing not only the mouse T1R2/T1R3 as mentioned above but also the rat homologs (Sigoillot et al. 2012). In mice, the sweet-suppressing effect of gurmarin has been observed to differ among strains (Ninomiya and Imoto 1995). However, polymorphisms in the mouse T1R3 gene were found to be not associated with strain differences in gurmarin sensitivity (Sanematsu et al. 2005).

The concentrations required to block sweet responses in vitro corresponded reasonably well to those that are effective

in vivo (Margolskee et al. 2007; Sigoillot et al. 2012). In contrast, responses of cells expressing the human T1R2/T1R3 remained unaffected. Thus, sequence differences in T1R2/T1R3 seem to be the basis for the observed species differences in taste sensitivity towards gurmarin and provide additional evidence for the sweet taste receptor being the target for gurmarin's suppressive effect on sweet taste.

Several authors have interpreted the inability of gurmarin to inhibit sweet taste responses recorded from the glossopharyngeal nerve as indication for the existence of at least two independent sweet detection pathways, one of which is sensitive to gurmarin and another one which is insensitive (Lemon et al. 2003; Ninomiya and Imoto 1995; Ninomiya et al. 1999; Yasumatsu et al. 2007). Recent findings suggest that, in situ, gurmarin sensitivity is linked to the coupling of T1R2/T1R3 to the taste cell-specific G protein α subunit gustducin (Ohkuri et al. 2009; Shigemura et al. 2008; Yasumatsu et al. 2009). The type of G protein-coupled to an activated GPCR has been reported to play a role in determining its specific response profile (Gudermann et al. 1996; Kenakin 2003). Whether this scenario holds true for T1R2/T1R3 and could underlie the molecular mechanism for gurmarin sensitivity remains to be seen. Furthermore, the unusually long-lasting blockade of rodent sweet taste responses by gurmarin suggests mechanisms of action that differ from simple competitive or allosteric inhibition.

Although the molecular mechanism of gurmarin inhibition is not known, at least two potential models of binding sites for gurmarin can be proposed (Fig. 7). Firstly, it has been demonstrated that the structural dynamics of the VFT of class C receptors is critical for their activation (Kniazeff et al. 2011; Pin et al. 2003). Based on the well-characterized mGluR₁ receptor, rodent T1R2/T1R3 may also oscillate between an opened and a closed conformation in the absence of bound ligand, where ligand binding stabilizes the closed state. One might assume that gurmarin binds VFT of rodent T1R2/T1R3 stabilizing the receptor resting state. Given the suppressive effect of gurmarin on rat taste nerve responses to mixtures of monopotassium glutamate or L-(-)-2-amino-4-phosphonobutyric acid with 5'-inosine monophosphate (IMP) (Sako and Yamamoto 1999), compounds that activate the T1R1/T1R3 umami receptor, one might conclude that the inhibitor acts on the common subunit of the sweet and umami taste receptor, T1R3. Hence, gurmarin may bind within the cleft of the closed form of the VFT of T1R3 (Fig. 7a), preventing closure of T1R2 or T1R1 VFT, thus blocking receptor activation. However, gurmarin may inhibit rodent T1R2/T1R3 in a second way. It has been demonstrated that relative movement of extracellular domains of GABA_B receptor VFT dimer is required for activation (Rondard et al. 2008). Figure 7b illustrates a second putative binding site for gurmarin, located at the dimerization interface of T1R2/T1R3. According to this

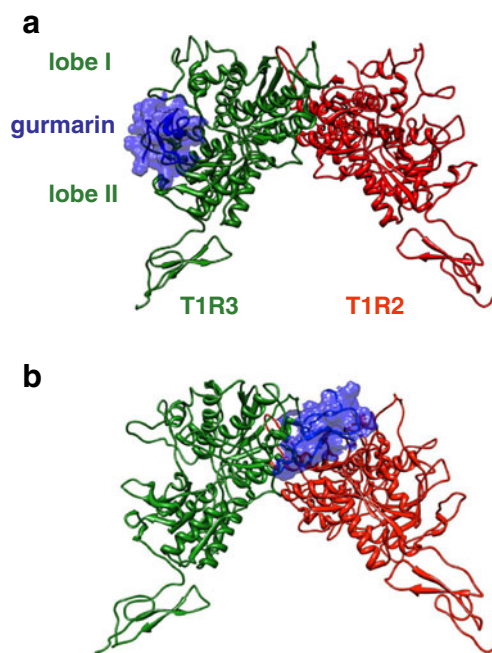


Fig. 7 Two possible models of the rat sweet receptor with bound gurmarin. Rodent T1R2/T1R3 heteromers are shown as *ribbon models*. T1R2 subunits are colored in *red*, whereas those of T1R3 are shown in *green*. Gurmarin is represented in *blue*. **a** Gurmarin binds to the open VFT of T1R3 in the cavity made by lobes I and II. **b** Binding of gurmarin at the interface of dimerization between rodent T1R2 and T1R3. The figures were prepared using UCSF Chimera software (Pettersen et al. 2004)

binding site, inhibition may result from the attachment of gurmarin to the subunit interface, preventing receptor activation by agonist sweeteners. Interestingly, the dimerization interface is composed of a large patch of hydrophobic residues in both subunits (Rondard et al. 2008), which could interact with the hydrophobic patch of gurmarin (Ota et al. 1998). Alternatively, although less likely, gurmarin may bind to the T1R3 TM, as previously observed with lactisole (Jiang et al. 2005a). Site-directed mutagenesis of amino acids present in these two putative gurmarin binding sites would be necessary to confirm all of these hypotheses. Experimental elucidation of the gurmarin inhibitory mechanism will significantly further our understanding of the processes involved in sweet taste receptor activation and signaling.

Other sweet taste inhibitors

Lactisole

Lactisole, the sodium salt of 2-(4-methoxyphenol) propionic acid, is a selective competitive inhibitor of human sweet taste perception (Schiffman et al. 1999). Approved as a food additive in the USA, it is employed in sweet products, such as jellies, to take advantage of the high sugar content required for the desired texture of the product in the absence of excessive

sweetness (LaBell 1989). Lactisole has also been successfully used as a research reagent to investigate the mechanisms underlying sweet taste perception, e.g., the supposed multiplicity of sweet taste receptors prior to the discovery of the T1Rs (Schiffman et al. 1999). In human psychophysiological tests, lactisole comprehensively blocks the sweet taste elicited by saccharides and other sweeteners (Schiffman et al. 1999; Ide et al. 2009). The inhibitory effect of lactisole is seen in humans and other primates. However, the substance has been shown to be ineffective in behavioral tests with rodents (Sclafani and Perez 1997). Guided by this interspecies difference, the molecular target of lactisole at the human sweet taste receptor was identified in the TM allosteric site of T1R3. Interestingly, this binding site (Fig. 8a) partially overlaps those of the sweet agonists NHDC and cyclamate (Jiang et al. 2005a, b; Winnig et al. 2005, 2007; Xu et al. 2004).

The human sweet taste receptor possesses constitutive activity. In absence of cognate agonists, it resides in a dynamic equilibrium between resting and active conformations (Galindo-Cuspinera et al. 2006; Sainz et al. 2007). Constitutive activity of GPCRs becomes apparent in elevated basal G protein activation and increased cytosolic calcium levels, both of which have been demonstrated in cells functionally expressing human T1R2/T1R3 (Fig. 8b) (Sainz et al. 2007; Galindo-Cuspinera et al. 2006). Lactisole acts as an inverse agonist shifting the equilibrium of the human sweet taste receptor towards the inactive conformation. As a result, basal G protein activation is diminished, and

intracellular calcium levels are decreased (Fig. 8b) (Galindo-Cuspinera et al. 2006). This scenario explains the global effect of lactisole inhibition on sweet taste, even on sweet compounds binding to distant sites in the VFT. Dissociation of lactisole from the receptor by rinsing with water shifts the equilibrium from the inhibited receptor state back to a constitutively active taste receptor. This off-response is perceived as an intensive sweet sensation known as sweet “water taste” (Fig. 8c) (Galindo-Cuspinera et al. 2006). The lactisole binding site is conserved in rat T1R3; however, the presence of a valine residue in position 738 seems to render this site inaccessible, and as a consequence, the rat sweet taste receptor is insensitive to inhibition by lactisole (Winnig et al. 2005).

Saccharin/acesulfame K

The two artificial sulfonamide sweeteners saccharin and acesulfame K both elicit complex taste impressions. At low concentrations, they are perceived as intensely sweet, while at higher concentrations, increasing bitter and metallic off-tastes become apparent (Schiffman and Gatlin 1993). The bitter component is intrinsic to both compounds and is caused by the ability of the sulfonamides to activate two bitter taste receptors, human T2R31 and T2R43 (Kuhn et al. 2004; Pronin et al. 2004; Roudnitzky et al. 2011). In addition, the perceived sweetness of saccharin and acesulfame K declines at concentrations beyond 6.25 mM (Galindo-Cuspinera et al.

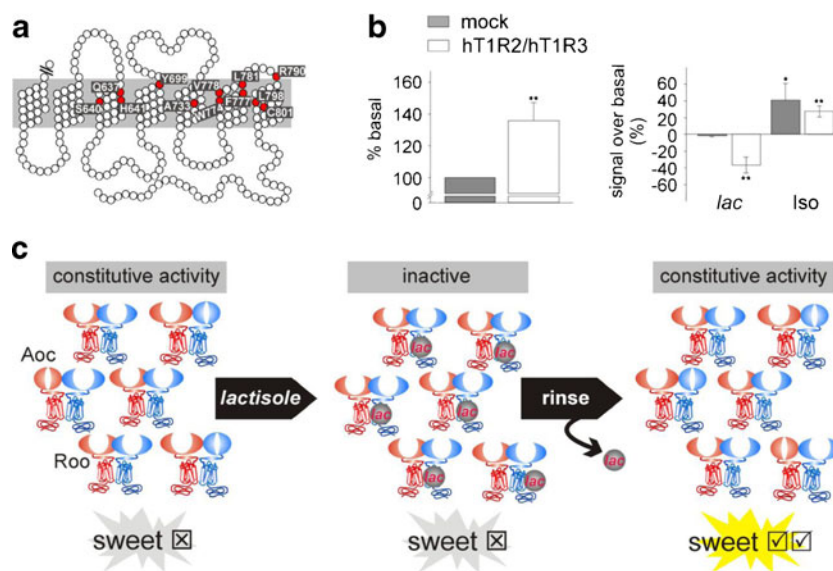


Fig. 8 Molecular mechanism of the inhibition of the human sweet taste receptor by lactisole. **a** Residues in the T1R3 TM which mediate the interaction with lactisole. **b** Increased basal GTP γ S³⁵ binding in T1R2/T1R3-expressing HEK293 cells (*left panel*) indicates constitutive activity of the human sweet taste receptor. In the presence of lactisole (*lac*), basal GTP γ S³⁵ binding is significantly reduced subject to T1R2/T1R3 (*right panel*). The andrenoreceptor agonist isoproterenol significantly elevates GTP γ S³⁵ binding over basal levels in

receptor-expressing and control cells. *One asterisk* denotes $p < 0.05$, and *two asterisks* denote $p < 0.01$ (T test). **c** Proposed mechanism underlying the inhibition of the human sweet taste receptor by the inverse agonist lactisole and subsequent gustatory sensations. Schemata of the T1R2/T1R3-heteromer represent the receptor activity state. *Roo* resting/inactive state with both subunit VFTs in open conformation. *Aoc* active state with one subunit VFT in open and one in closed conformation. Modified from Winnig (2006)

2006). This effect originates from the inhibition of human T1R2/T1R3 by sulfonamide agonists, which, at high concentrations, bind to a second low-affinity allosteric site. Similar to lactisole inhibition of T1R2/T1R3, the allosteric binding of saccharin or acesulfame K might shift receptor equilibrium to the inactive state, thus blocking receptor activation and, accordingly, sweet taste perception. Consequently, rinsing the sulfonamides from the T1R2/T1R3-expressing cells reactivates the receptor and induces a sweet water taste off-response similar to that experienced with lactisole (Galindo-Cuspinera et al. 2006). The inhibitory allosteric site of action for saccharin and acesulfame K has been mapped to human T1R3 TM, distant from the receptor-activating orthosteric site located in the VFT of T1R2 (Galindo-Cuspinera et al. 2006; Masuda et al. 2012).

Thus, sweet water taste perception is associated with sweet taste-blocking compounds. It is based on the immediate release of T1R2/T1R3 from inhibition upon removal of the negative allosteric modulator and the subsequent revival of receptor activity.

Further perspectives

Consumer preferences for the combination of fatty-sweet and fatty-savory-tasting foods are inadvertently leading to a rise in prevalence and incidence of diabetes mellitus and hyperlipidemia. In the context of dietary needs, as well as over- and malnutrition, it is worthwhile to investigate sweet and umami inhibition. The role of T1R2/T1R3 in gastrointestinal physiology, specifically in glucose absorption, bears some therapeutic potential for small molecule sweet inhibitors. Reduced glucose absorption and therefore reduced calorie uptake by such compounds would offer an alternative to treat obesity and its associated diseases. Importantly, in this setting, these sweet-taste-inhibiting drugs would reach their target from the luminal side, not from the systemic side, minimizing potential side effects.

Similarly, plant-derived sweetness inhibitors, including gymnemic acids from *G. sylvestre* R. Br. (Liu et al. 1992) and ziziphin from *Z. jujuba* (Meiselman et al. 1976), have been proposed as alternative therapies for lowering high blood sugar levels and treating obesity. For example, *G. sylvestre* has been used in the ayurvedic medicine to treat diabetes mellitus (Hardy et al. 2001; Warren et al. 1969). In Japan, it has been traditionally observed that drinking teas made from *G. sylvestre* leaves helps to control body weight. However, the clinical efficacy of *G. sylvestre* use has only been supported by a small number of nonrandomized, open-label trials (Leach 2007). Moreover, a recent study has reported a case of drug-induced liver injury in a patient who was treated with *G. sylvestre* for diabetes mellitus (Shiyovich et al. 2010).

Gurmarin is emerging as another potential therapeutic candidate for the treatment of hyperlipidemia and type II diabetes. It is widely recognized that peptides or polypeptides make excellent drug leads because they have evolved to be highly specific and potent for their receptors and typically have low toxicity. In addition, the cysteine knot structural motif of gurmarin would confer to this polypeptide a particularly high degree of thermodynamic and biological stability (Craik et al. 2001). As gurmarin is only active on rodent T1R2/T1R3 and not on the human taste receptor, one possibility would be to use site-directed mutagenesis or directed evolution (Bonsor and Sundberg 2011) in the development of gurmarin-based therapeutics that exhibit a high affinity and specificity for human T1R2/T1R3. The use of directed evolution coupled to structural and energetic analysis of gurmarin–receptor interactions may be the key to achieving this goal.

The research into the mechanisms of sweetness inhibition and the possible design of new sweetness inhibitors undoubtedly represents an area of interest for pharmaceutical and food development industries. Hopefully, such therapeutic opportunities will, vice versa, promote basic research into elucidating the mechanistic details of sweet and umami taste receptor activation and inhibition.

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