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TAS1 receptors. An overview of their functions, expression and genetic variations

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Abstract Heteromeric G protein-coupled receptors are essential in taste transduction, a characteristic important for vertebrates. Type 1 taste receptors mediate sweet and umami sensing via two heterodimers: TAS1R2/TAS1R2 and TAS1R1/TAS1R3. Evidently, these heterodimers are expressed in taste buds, but also in several other tissues like the gastrointestinal tract, bone, pancreas and bladder. Because of its role in transducing the sweet taste, there have been plenty of investigations regarding genetic variations associated with obesity or dental caries.

Keywords: G protein-coupled receptors, sweet taste, receptor variants

Introduction

Heteromeric G protein-coupled receptors (GPCRs) play a major role in taste transduction. The perception of different taste qualities in vertebrates is essential for avoiding the intake of noxious foods and seeking more nutritious ones. Vertebrates and especially mammals can differentiate between four basic tastes: salty, sour, sweet and bitter with two additional flavor sensations: umami and kokumi. Except for salty and sour, all other tastes are generated by GPCRs activation. Type 1 taste receptors (TAS1Rs) mediate sweet and umami sensing via two heterodimers: TAS1R2/TAS1R3 and TAS1R1/TAS1R3, respectively (Tomás et al., 2016). Due to their importance in feeding behavior, TAS1Rs are activated by a large variety of dietary compounds which bind to these receptors with different affinities. TAS1R2/TAS1R3 proteins are targeted for developing new low-calorie and high-potency artificial sweeteners designed to replace sugars. For this purpose, *in vitro*, *in silico* and psychophysical studies are commonly used to identify the binding properties of ligands and to investigate which amino acid residues are necessary for their interactions. Apart from their presence in taste buds, TAS1 receptors are also found in the gastrointestinal tract, pancreas, bladder, bone and adipose tissue.

In the central nervous system, TAS1R2/TAS1R3 heterodimers are expressed in neurons and epithelial cells of the choroid plexus (Ahmad and Dalziel, 2020). Recent screenings of the TAS1 gene variations were correlated with pathologies like obesity and dental caries, but also with food preferences across species and individuals. In this review we will discuss the structure, function, signaling and expression of TAS1R2/TAS1R3 receptors as well as their variants and physiological implications.

Structure and function of TAS1R2/TAS1R3 heterodimers

The proteins encoded by the TAS1R genes are G-protein coupled receptors with seven trans-membrane domains. These proteins form heterodimers in order to detect sweet (TAS1R2/TAS1R3) and umami (TAS1R1/TAS1R3) tastes (Tomás et al., 2016). However, some studies showed that both human TAS1R2 and mouse TAS1R3 can form functional homodimers in heterologous expression systems and in pancreatic β-cells respectively (Belloir et al., 2021). Spectroscopic studies revealed that the TAS1R2 subunit consists of 66% α-helix and 18% β-sheet. This subunit showed a very high affinity for the artificial sweetener neotame, followed by sucralose and acesulfame-K (Belloir et al., 2021).

The large extracellular domain of TAS1R2/TAS1R3 consists of a Venus flytrap module (VFD) and a cysteine-rich domain (CRD), both important in allosteric modulations of the receptors. Multiple ligands activate these receptors by binding to different sites (Cui et al. 2006).

TAS1R2 was shown to interact with the Ca^{2+} and integrin-binding protein (CIB1) through the methionine residue located in position 818. Both proteins are present in human taste cells where alterations of the CIB1 levels modify the TAS1R2/TAS1R3 heterodimer response to ligands. This effect may be attributed to the interaction between the C-terminal domain of TAS1R2 and CIB1 which influences the heterodimer level of expression on the plasma membrane (Hennigs et al., 2008).

The canonical dimer TAS1R2/TAS1R3 is also sensitive to some amino acids, depending on their chirality. D-His, D-Trp, D-Phe and D-Leu, D-Val, D-Ile, D-Ala and Gly activate the dimer and are perceived as sweet, but the L-forms of these amino acids do not activate the receptor. It is not yet clear what causes this stereoselectivity (Bassoli et al., 2014), but there seems to be no discrepancy between L- and D-glucose. Both configurations bind to the same region of TAS1R2 and no point mutations have been identified which would affect one and not the other. Moreover, the subjective perception in sweet taste tests revealed no significant difference between the enantiomers (Dubovski et al., 2022).

In silico simulations showed that sucrose has a binding site mediated by hydrogen bonds close to the D142 and E302 residues. Allosteric ligands such as lactisole interact with S807 and S819 of TAS1R2 and TAS1R3 respectively. These interactions remain stable during molecular dynamics and modulate the receptor activity by binding to the bottom half of the VFD domain. The structural changes induced by the interaction with the allosteric ligands and the structural changes of the VFD occur in the cysteine rich domain 3, which is linked to the transmembrane domain 3 (TMD3) (Kim et al., 2017). Docking studies showed that various sweeteners activate the sweet receptors independent of the VFD of TAS1R2 (VFD2). Sucralose has a higher affinity for VFD2, but can also bind to VFD of TAS1R3 (VFD3), where it interacts with S146, S147, E301 and F274. Likewise, saccharin and acesulfame K can also bind to TMD3 and VFD3. In the same study, the presence of both acesulfame K and sucralose was shown to have a synergistic effect on the activation of the receptor which is probably also reflected in the sweetness perception (Jang et al., 2021).

The intensity of sweetness sensation is also correlated with the affinity of the compound on TAS1R2/TAS1R3 receptors. Sweeteners with an EC_{50} less than 1 mM such as saccharin, are perceived as sweeter, compared to D-fructose which has an EC_{50} higher than 1 mM (Choi et al., 2021). Moreover, TAS1R2/TAS1R3 receptors are activated by heavy water which, interestingly is also perceived as sweet (Ben Abu et al., 2021). Temperature was also found to influence the sensation of sweetness. In one study, lactisole, an inverse agonist of TAS1R2/TAS1R3, was orally administered to participants who later dipped their tongues in flowing water at different temperatures. The perceived sweetness was reported to be maximal in the 20°-35°C range with no significant differences within this interval. The subjects did not report sensing any other tastes during the experiment (Nachtigal & Green, 2020). After washing away the inverse agonist, TAS1R2/TAS1R3 receptors return to their active state, thus inducing the sensation of sweetness. Na-saccharin and acesulfame-K were found to cause the same effect as lactisole when washed away from the tongue. Some artificial sweeteners lose their ability to produce the desired sensation at higher concentrations. These mechanisms can be explained by the allosteric properties of the receptors and the different binding sites of the ligands at high and low concentrations. This supplementary binding induces loss of sweet perception (Alvarado et al., 2017; Galindo-Cuspinera et al., 2006). The activity of the TAS1R2/TAS1R3 agonist, miraculin, is dependent on both intracellular and extracellular pH with lower extracellular pH enhancing the sweetness sensation. Miraculin was found to interact with several histidine residues of the amino terminal domain (Sanematsu et al., 2016).

Expression and signaling

1. Expression

The role of TAS1 receptors was mainly characterized in taste sensing, but recent studies found the expression of these proteins to play multiple roles in different tissues. The expression levels of Tas1r1 and Tas1r2 genes were elevated in the hypothalamus but not the cortex of food-deprived mice, while the expression of Tas1r3 was not affected by the dietary state of the rodents (Ren et al., 2009). Another study found increased levels of mRNA coding for TAS1R2 and TAS1R3 in taste buds of mice which were fed a high-fat diet and were given asperuloside, a compound with anti-obesity potential. Neither the high-fat diet, nor asperuloside alone induced any mRNA changes. The investigators did not look for mRNA modifications in the hypothalamus or cortex (Ishaq et al., 2021). Bacterial lipopolysaccharides (LPS) were also found to influence gene transcription of taste receptors in taste cells. Even though no particular effect was observed after acute treatment, LPS application for seven days had a significant effect in decreasing the Tas1r2 and Tas1r3 mRNA levels in mice (Zhu et al., 2014).

In an apolipoprotein E (a fat-binding protein, ApoE) knock-out mouse model, TAS1R3 ablation improved the overall health condition when animals were fed a high-fat diet. Atherosclerotic plaque in the aorta, liver lipid accumulations, total body weight, circulating glucose and insulin levels were greatly reduced in the double knock-

out, compared to ApoE-/- TAS1R3+/+ mice (Shojaat et al., 2020).

Even though these receptors have been associated with various metabolic disorders, TAS1R3 expression was not modified in the jejunal tissue of women with obesity or metabolic syndrome (Bertran et al., 2021). Several studies identified the heterodimeric receptor TAS1R2/TAS1R3 in the human small intestine, but the exact cellular location is still unclear. There is yet no evidence of the sweet taste receptors in the CaCo-2 enterocytes cell line, but application of an equimolar mix of D-glucose and D-fructose was shown to stimulate glucose transport in these cells (O'Brien & Corpe, 2016). The sugar 3-deoxyglucosone was found to decrease the expression of TAS1R2 and TAS1R3 in the duodenum and colon of rats. This effect may be mediated by the increase of glucagon-like peptide 1 (GLP1) secretion. This is supported by the fact that, in glucose free conditions, 3-deoxyglucosone determines GLP1 secretion which is inhibited by lactisole (Song et al., 2019; Zhang et al., 2016).

An investigation of fungiform and circumvallate papillae from rhesus macaques found that these cells can be classified in three subtypes based on the expression of TAS1Rs and their associated G-proteins. There are cells that express TAS1R1 and TAS1R3, TAS1R2 and TAS1R3 and only TAS1R3. The same study found only α-gustducin 3 (GNAT3) and not α-gustducin 4 (GNAT4), to be present in the cells expressing TAS1 proteins. This suggests that GNAT3 is a key factor in the transduction of sweet and umami tastes in macaques (Ishimaru et al., 2012).

The mRNA of TAS1R1 and TAS1R2 was identified in the gastrointestinal tract from various carnivorous species of fish. The levels of these mRNAs were up- and down-regulated by L-leucine, L-proline and L-valine in different segments of the gastrointestinal tract. The mechanisms of these alterations are still unclear (Calo et al., 2021; Polakof & Soengas, 2013). In LacZ-reporter mice, TAS1R2 was identified in taste buds, duodenum, jejunum, ileum and colon. There was no signal indicative of TAS1 receptors in the circumvallate or fungiform papillae during the embryonic stage. The receptors were detectable at P0 and their expression increased during test bud maturation (Iwatsuki et al., 2010). Another study also identified the presence of TAS1R3 transcript in the stomach and all the TAS1 transcripts along the intestine. TAS1R1 expression was found to be time-dependent, with a 31% increase between 7 AM and 7 PM. This alteration was not observed for the other TAS1 genes, nor in the other gastrointestinal segments (O'Brien et al., 2018). Analysis of the equine small intestine revealed the presence of TAS1R1 and TAS1R2, with similar expression levels across all segments, although the proximal regions were slightly more enriched than the distal ones. The presence of the sweet receptors in these tissues seem to play a role in glucose metabolism, regulating the expression of the intestinal glucose cotransporter, SGLT1 (Daly et al., 2012).

TAS1R3 was also found in the murine osteoblast lineage, including osteoclasts. A murine TAS1R3 loss-of-function mutation led to increased bone mass due to the inability of osteoclasts to resorb bone matter (Eaton et al., 2018). Various sweeteners induce calcium release in tanycytes, the epidermal cells that coat the third and part of the fourth brain ventricles, where TAS1R2 and TAS1R3 mRNA was identified by RT-PCR. Additionally, TAS1R2 null mice revealed a population of tanycytes that did not respond to glucose while another population preserved its sensibility. This suggests that two independent mechanisms are involved in glucose sensing (Benford et al., 2017). One study identified the presence of TAS1R1, TAS1R2 and TAS1R3 mRNAs in murine hypothalamus, hippocampus and cortex, the highest level being observed in the hypothalamus. The authors concluded that these proteins are preferentially expressed in neurons, but are also found in the choroid plexus and periventricular areas (Ren et al., 2009). The expression in the choroid plexus was later confined to the cytoplasm and plasma membrane of the epithelial cells, where both heterodimers were identified, along with their associated G-protein, α-gustducin (Tomás et al., 2016).

1. Signaling

The intracellular signaling pathway of TAS1R activation is, in part, shared by the TAS2 receptors. After ligand binding to TAS1R2/TAS1R3 heterodimers, the β3γ13-subunits of the G-protein induce calcium release from the endoplasmic reticulum (ER) via phospholipase Cβ2 (PLCβ2) and inositol 1,4,5-trisphosphate (IP3) pathway (Zhang et al., 2007). The release of Ca^{2+} opens the transient receptor potential melastatin 4 and 5 (TRPM4/5) ion channels generating action potentials followed by ATP release (Banik et al., 2018). At the same time, the G-protein α-gustducin subunit activates adenylate cyclase (AC) which elevates intracellular cAMP. The rise in cAMP levels enhances protein kinase A (PKA) activity which depolarizes the membrane through the inhibition of voltage-dependent potassium channels (VDKC) (Ohmoto et al., 2006). Sodium influx through voltage dependent sodium channels (VDSC) leads to further depolarization having a synergistic effect on action potential discharge. Thus, both G-protein α and βγ subunits contribute to intracellular Ca^{2+} and Na^{+} increase followed by membrane depolarization. The end result is ATP exocytosis through calcium homeostasis modulator 1 and 3 (CALHM1/3) (Fig 1) (Ma et al., 2018). Extracellular ATP binds to the P2XR expressed on the afferent cranial nerve fibers, transmitting the taste

sensation to the gustatory cortex (Margolskee, 2002). However, other Gα subunits were also identified in taste cells that express the sweet taste receptors. The mRNA for Gαi−2, Gαi−3, Gαs and Gαq was found in taste tissues from rats and gustducin knock-out mice seem to retain some of their sweet-sensing ability (Danilova et al., 2006). Multiple investigations point to an important role of this protein in taste transduction, but different interacting effectors may coexist in the same cell types.

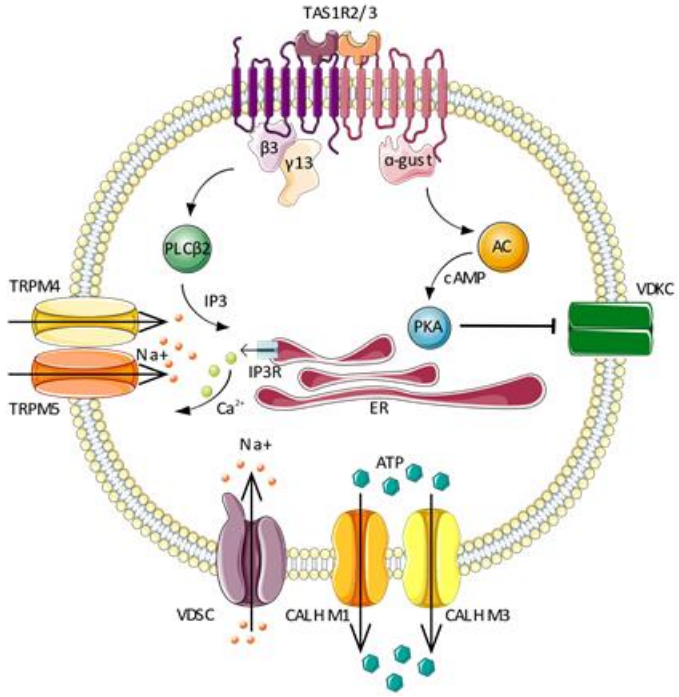

Fig 1. The signaling pathway of the TAS1R heterodimer activation. When a ligand binds to the TAS1R2-TAS1R3 dimer, the G-protein β3γ13-subunits subunits induce Ca^{2+} release from the ER via the PLCβ2 and IP3 pathway. The rise in intracellular calcium induces TRPM4 and TRPM5 activation which leads to sodium influx and ATP release via CALHM1 and CALHM3. Further sodium influx is mediated by VDSCs The α-gustducin subunit induces an increase in cAMP and the activation of PKA, which phosphorylates and thus inhibits VDKCs

Evolution and variants

1. Human variants

An analysis of diversity in the human population found that most of the variation in sweet and umami receptors appears due to changes in TAS1R1 and TAS1R2, while TAS1R3 is the most conserved among humans (Kim et al., 2006). The TAS1R1 variant, rs17492553 and the TAS1R2 variants, rs3935570 and rs4920566 were investigated in Brazilian children and preadolescents. Only the latter group had a borderline association of the rs17492553 with dental caries (Arid et al., 2020). In a study in which 647 Italian people were screened, the rs3935570 variant was also associated with dental damage (Robino et al., 2015). One hedonic study found the TAS1R2 variants to be positively associated with vodka preference, while only rs4920566 was associated with red wine preference in some Caucasian populations (Pirastu et al., 2012). Another variant of TAS1R2, rs9701796, was associated with a higher preference for sweetness and with the amount of sugar intake of the variant carrier (Chamoun et al., 2018). No correlation was found between this variant and the energy and macronutrient intake of patients one year after Roux-en-Y gastric bypass surgery (Novais et al., 2021).

In a study of Swedish middle-aged people, the TAS1R2 genotype was only moderately associated with dietary habits. The only effect was observed in people with a body mass index larger than 25, where the ones with the T allele had a greater proportion of carbohydrates in their diet (Habberstad et al., 2017). Moreover, the Ile191Val TAS1R2 variant (rs35874116) was associated with more carbohydrate consumption in two distinct populations of overweight and obese individuals (Eny et al., 2010). Further investigations of this variant in people with various BMIs found that this polymorphism was not correlated with weight (Koc et al., 2021). The same polymorphism was investigated in regards to dental caries. One study did not find any association between the variant and dental problems (Kulkarni et al., 2013), while another found marginal significance in children (Holla et al., 2015).

Variants of the Tas1r1 gene were shown to influence birth weight. Babies carrying the rs4908932 variant are on average 87 grams heavier at birth. Although it is yet unclear what the phenotypic consequences of this mutation are, the carriers seem to present a stronger methylation of the DNA in many tissues of the gastrointestinal tract (Farinella et al., 2021). The rs11261087 TAS1R2 variant was associated with pancreatic cancer in a study of almost 15 thousand people, while the normal variant is considered to offer protection from the development of this disease (Gentiluomo et al., 2019).

TAS1R variants also influence the sensitivity to sweet stimuli. A study found two single-nucleotide polymorphisms (SNPs) in a non-coding region, which influence the transcription rate of the gene. The alleles associated with lower sweet sensitivity in people also have a lower transcription level in a heterologous system. In terms of distribution, the study found that said variants have a rather wide distribution in the population (Fushan et al., 2009). Interestingly, people with a BMI over 25 which also carry a specific variant of TAS1R2 (the G allele of rs12033832) are less sensitive to sweet compounds, (i.e., they detect sweetness at higher concentrations of the substance). The effect was opposite for people with a lower BMI, which displayed a lower threshold for sweet sensing. The same effects were observed in connection to carbohydrate intake, where people with BMI>25 are eating more carbohydrates compared to the ones with lower BMI (Dias et al., 2015). These results were replicated in terms of carbohydrate intake, but not sweet sensitivity, in a study using different kinds of soups. Unfortunately, the study had a low

number of participants and did not take into account BMI differences (Han et al., 2017).

Apart from sweet preference tests, one study investigated the effect of TAS1R1 and TAS1R2 variants on sour taste preference, finding SNPs associated with people liking more or less sour solutions. The article did not find any molecular explanation for this strange characteristic, so the authors proposed an effect linked with other taste sensitivities (Eriksson et al., 2019).

2. Variants in evolution

Some amphibians have a duplicated Tas1r gene, but overall, there seems to be no correlation between feeding behavior and the status of the Tas1r genes in these species. There is no significant difference in the diets of anuras, even though their Tas1r genes can be functional, pseudogenized or completely absent (Zhong et al., 2021).

One study investigated the evolution of the Tas1r genes in reptiles, but found no correlation with their feeding behavior. The majority of snakes seem to have lost the umami and the sweet sensations, but for the rest of the species no clear pattern was observed. Most reptiles are insectivorous or carnivorous, but the Tas1r genes can either be intact, partially intact or completely turned into a pseudogene (Feng & Liang, 2018).

Variants of the TAS1 genes have also been studied in cows and correlated with growth characteristics. From 436 female cattle investigated, 17 SNPs were identified of which four were directly associated with hip and sacra heights and tallness (Zhang et al., 2012). Most birds including chickens seem not to sense sweetness, considering the absence of an ortholog for the human TAS1R2 (Lagerström et al., 2006). However, hummingbirds can sense sugars through a TAS1R1/TAS1R3 umami heterodimer mutation (Baldwin et al., 2014).

A study concerning cats found that the mechanism necessary for sweet taste perception is absent. The Tas1r3 gene is expressed and most probably functional at the protein level, but Tas1r2 is transformed into a pseudogene. Cats, tigers and cheetahs have the same 247 base pair deletion in exon 3 and a stop codon in exon 4 (Li et al., 2005). There is a great variation in carnivores regarding the status of their Tas1r2 gene. In some species the gene is functional while in others it was mutated into a pseudogene. The loss of function mutations were identified in different locations for all the species analyzed, and therefore the authors concluded that these changes appeared independently. Some of the investigated species were the spotted hyena, the sea lion, the small-clawed otter and the bottlenose dolphin (Jiang et al., 2012).

Insectivorous and frugivorous bats were found to differ in their preferences to sweet compounds. Unsurprisingly, the frugivorous bats preferred solutions containing natural sugars, while the insectivorous bats did not. The same group further investigated the expression levels of the Tas1r2 and Tas1r3 genes, in the tissues where they were found in the respective bats. Finally, various *in vitro* experiments were conducted in order to identify why the insectivorous bats lost their ability to perceive sweetness. The researchers concluded that the VFD of the TAS1R3 version from insectivorous bats is modified, making the protein unresponsive to sugars (Jiao et al., 2021). In contrast, another study investigating 42 species of bats found that the Tas1r2 gene is under the same level of purifying selection in both fruit and insect eating bats. Purifying selection refers to the gradual removal of alleles that are not advantageous. This analysis suggests that the structure and function of the protein is preserved among bat species regardless of their diet. The same investigators also found that vampire bats have their taste related genes degenerated to pseudogenes (Zhao et al., 2010).

There is also a difference concerning the molecules perceived as sweet by Old and New World monkeys. One study showed that the squirrel monkey TAS1R2/TAS1R3 heterodimer was not activated by the sweeteners aspartame, neotame and saccharin. The same study also confirmed that the TAS1R2 subunit is needed for saccharin sensitivity (Cai et al., 2016). The amino acids responsible for these interactions are placed in positions 40 and 142. In these positions, the human isoform has a serine and, respectively an aspartate residue, while the squirrel monkey variant has a threonine and a glutamate residue. This difference induces a conformational change which influences Y103 and N143, thus reducing the size of the binding site required for aspartame interaction (Liu et al., 2011).

CONCLUSIONS

In this review we aimed to describe the expression and function of the sweet taste receptors along with their role in evolution and genetic variations. The importance of these receptors in diet selection was well investigated especially with the development of low-calorie sweeteners. However, the recent discovery of their functional expression in a large number of extra-oral tissues indicates a plethora of physiological roles in the organism's metabolism and homeostasis. Further investigations of these receptors may lead to a better design for artificial sweeteners and a deeper understanding of glucose metabolism. Furthermore, by tracing the genetic variations throughout evolution and between different species, we may be able to infer how environmental constraints are reflected by dietary changes.

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