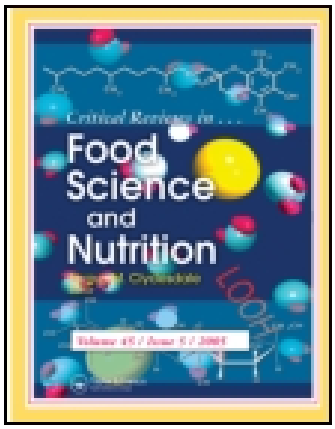

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Molecular Basis of Taste Sense: Involvement of GPCR Receptors

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Taste perception is one of the senses crucial for many organisms. There are five basic tastes, i.e., sweet, bitter, salty, sour, and umami, and it is suggested that the taste of fat should be included in this list. This paper reviews the current state of knowledge about the involvement of G protein coupled receptors (GPCRs) in taste sensing and intracellular signaling. GPCR receptors are focal point of interest for pharmaceutical industry. However, their ability to interact with a variety of taste substances makes these receptors interesting target for food and nutrient companies.

Keywords Taste sensing, GPCR, signal transduction, oral

INTRODUCTION

G protein coupled receptors (GPCRs) are encoded by one of the largest groups of genes found in human genome (Fredriksson et al., 2003). They are members of highly conserved membrane proteins present in all eukaryotes with homologs found in bacteria. GPCR receptors are involved in many different biological processes, such as neurotransmission, chemoattraction, the functioning of sense organs (taste, smell, and sight), as well as the regulation of appetite, blood pressure, digestion, and reproduction (Ferguson and Caron, 1998; Nuez Veulens and Rodríguez, 2009).

GPCR receptors are known as seven trans-membrane receptors (7TM) as their common feature is the presence of seven α-helical trans-membrane domains (TM 1-7) combined with three extracellular loops (els) and three intracellular loops (ils) (Bockaert and Pin, 1999). N-terminal fragment is located outside the cell, while the C-terminal inside the cell (Karnik et al., 2003). Ligand binding site is located in the transmembrane and extracellular regions, while the G protein interaction site is located in the third intracellular loop and C-terminal region (Kristiansen, 2004; Nuez Veulens and Rodríguez, 2009). Due to the presence of the structural characteristics, GPCR receptors are divided into four classes: A, B, C, and frizzled/smoothened (Figure 1; Fredholm et al., 2007). GPCR receptors are characterized by great diversity of ligands, among which are: flavors and fragrances, pheromones, hormones, neurotransmitters, and even light (Fredriksson et al., 2003). GPCR receptors, for which no natural ligand has been found so far are called orphan receptors (Hakak et al., 2003).

GPCR receptors are coupled to heterotrimeric G proteins and are activated by ligand binding. Functional G protein is composed of three subunits: Gα having a nucleotide-binding site and the activity of GTPase, and Gβ and Gγ forming a heterodimer (Hur and Kim, 2002). Gα subunit in its inactive state contains guanosine diphosphate (GDP), while in active state it contains guanosine triphosphate (GTP). Gα and the G$\beta\gamma$ form heterocomplex that allows anchoring to the cell membrane. G$\beta\gamma$ heterodimer is involved in Gα coupling to GPCR receptors, but also slows down spontaneous release of GDP, thus acting as an inhibitor of GDP nucleotide dissociation factor (GDI, guanine nucleotide dissociation inhibitor; McCudden et al., 2005).

It was observed that GPCR receptors interact not only with G proteins, but also with proteins called arrestins and proteins containing PDZ motif (Post synaptic density protein, Drosophila disc large tumor suppressor, Zonula occludens-1 protein). Arrestins bind to the GPCR receptors phosphorylated by GPCR regulatory kinases (GRKs), which leads to their desensitization through the dissociation of G proteins and GPCR receptors (Bockaert and Pin, 1999).

SENSE OF TASTE

Ability to perceive, recognize, and respond to stimuli is essential for the survival of organisms. Gustatory system has two primary functions, i.e., recognition of essential nutrients and

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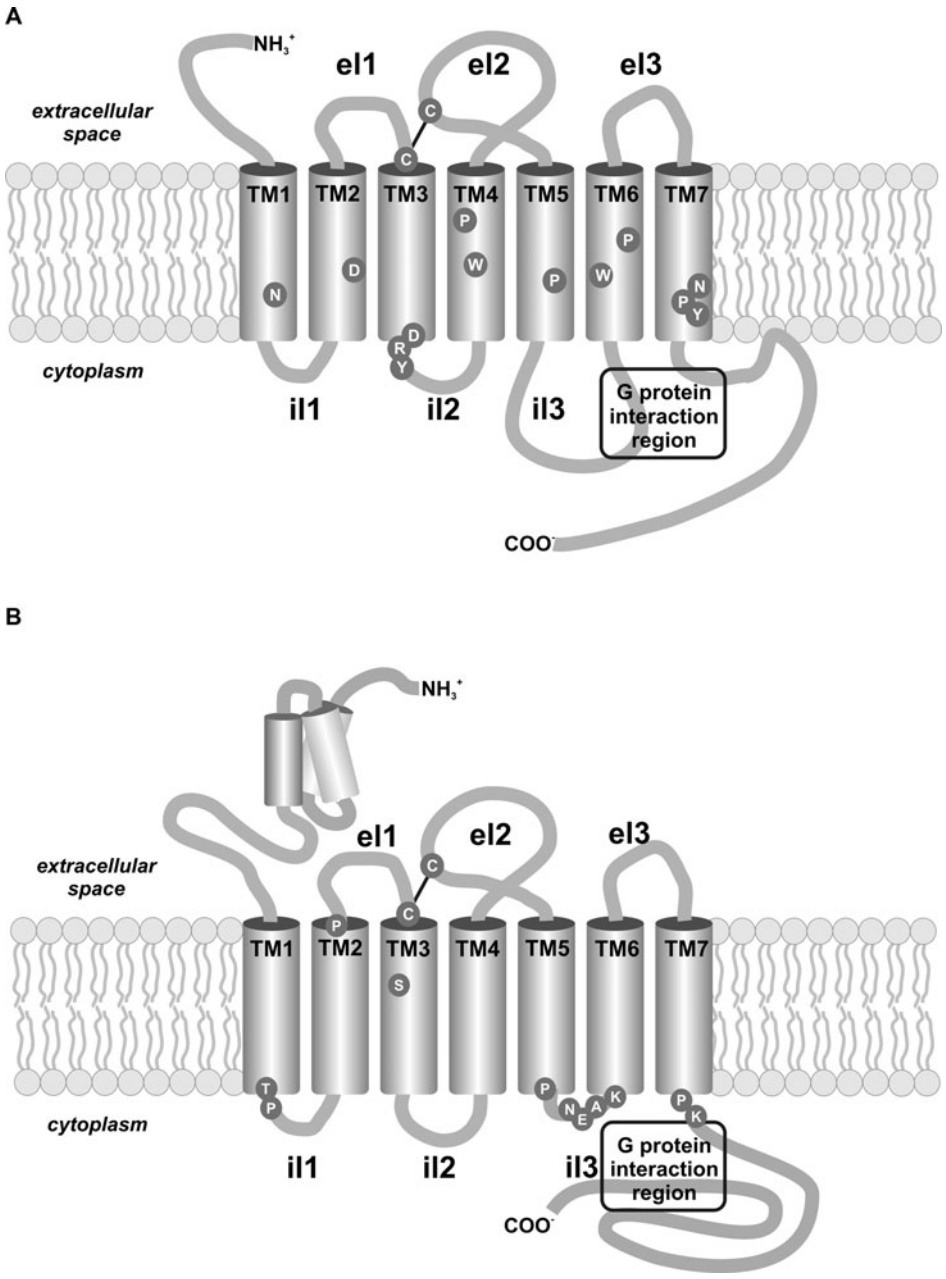

Figure 1 Diagrams of GPCR receptors from class A (A) and class C (B). TM 1-7 – transmembrane domains, el 1-3 – extracellular loops, il 1-3 – intracellular loops; grey circles conserved amino acid residues.

activation of digestive system, as well as protection against poisonous or otherwise harmful compounds in ingested food. Taste is one of the basic senses available for organisms (Andres-Barquin and Conte, 2004). The sense of taste belongs to the chemical senses through which we receive information about the presence of chemicals in food. To taste a substance, it must be dissolved in water (saliva).

Humans recognize five basic tastes: sweet, bitter, salty, sour, and umami (Matsunami et al., 2000; Yoshida et al., 2005). Recent studies indicate that human is able to detect the sixth taste, i.e., the taste of fat by reaction to free fatty acids (FFAs) present in food (Stewart et al., 2010).

It was shown that GPCR receptors are involved in recognition of sweet, umami, and bitter tastes, as well as the taste of fat (Kitagawa et al., 2001; Huang et al., 2006; Khan and Besnard, 2009). The taste of salty and sour is detected by the action of sodium, potassium, or hydrogen ions on ion channels or ion exchangers in the taste buds (Mombaerts, 2004).

Human Taste Perception

Taste sensory system comprises taste papillae located on the tongue (Mombaerts, 2004). There are three types of taste

sensing structures: circumvallate papillae occurring in the middle and back of the tongue, foliate papillae located on the sides, and fungiform papillae occupying two-thirds of the front surface of the tongue (Sainz et al., 2001). Taste papilla is composed of taste buds grouped in structures resembling garlic cloves (Palmer, 2007). Circumvallate papillae consist of hundreds (in mice) to thousands (in humans), foliate papillae of dozens to hundreds, while fungiform papillae of only a couple of taste buds (Chandrashekar et al., 2006). Each of taste buds is made up of 50–150 specialized taste receptor cells (TRCs), which are transformed epithelial cells with some properties of the neurons, such as the ability of depolarization or the creation of synaptic connections (Gilbertson et al., 2000; Clapp et al., 2001; Mombaerts, 2004). TRC cells contain apical microvilli that assist in the interaction with the ligand (Kinnamon, 1996; Chandrashekar et al., 2006).

Taste buds are innervated by the hypoglossal nerve (back of tongue), a branch of the tympanic facial nerve cords (anterior surface of the tongue) and the vagus nerve (near the larynx) (Gilbertson et al., 2000). As a result of recognition of the respective ligand, TRC cells initiate signal transmitted by synaptic connection with sensory neurons that in turn transmit a signal to the brain stem and the thalamus (Mombaerts, 2004).

Recent molecular and functional studies have undermined the common belief that there is a so-called "taste map." Previously, it has been assumed that the tongue has a topographic organization and the taste buds of specific areas have preferences for different taste stimuli (Dulac, 2000). It turned out that each area of the tongue is capable of receiving all five tastes (Chandrashekar et al., 2006), because the taste buds are present in all areas of tongue, and each contains the TRC cells sensitive to different types of flavors (Dulac, 2000).

Taste Receptor Cells

TRC cells are divided into four types termed I, II, III, and IV (Breslin and Spector, 2008). Type I cells have some similarities to glial cells (Palmer, 2007). They express ecto-ATPase, which probably restricts the diffusive spread of adenosine-5'-triphosphate (ATP) inside the taste bud, limiting its operation to a smaller group of cells (Roper, 2007).

Type II cells, known as sensory receptor cells, are excited as a result of stimulation by taste ligands (Roper, 2007). It was observed that they express GPCR receptors involved in taste sensing, i.e., T1R and T2R taste receptors, and molecules involved in signal transduction of sweet, umami, and bitter taste (Clapp et al., 2006). Voltage-dependent sodium and potassium channels were found to be present in these cells (Palmer, 2007). It was shown that type II cells do not form typical synaptic connections, but stimulation with specific taste substances leads to the transmitter (ATP) release by pannexin or connexin channels (Roper, 2007; Ishimaru, 2009). It was observed that the released ATP acts on both, type III cells and the afferent nerves (Roper, 2007).

Type III cells are known as presynaptic cells due to the fact that they show ultrastructural features of synapses (Roper, 2007). They have enzymes for the aminergic neurotransmitters and proteins involved in vesicle release (Roberts et al., 2009). Type III cells express voltage-gated calcium channel NCAM molecule (neural cell adhesion molecule) and SNAP-25 protein (synaptosome-associated protein of 25 kDa). The latter is a soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex member, involved in vesicle fusion to the membrane during exocytosis. It was shown that type III cells form synapses with sensory nerves and release neurotransmitters: serotonin and norepinephrine (Palmer, 2007; Roberts et al., 2009). Type III cells in contrast to type II cells are not excited by stimulation with taste ligands (Roper, 2007).

TRCs known as type IV (basal) are located at the bottom of the taste buds and are considered to be progenitor cells for other types of TRC cells (Ishimaru, 2009).

Taste Sensing

The taste sensation is caused by the interaction of taste substances with receptors or ion channels located in apical membrane microvilli of the TRC cells, which results in intracellular signal manifested by generation of secondary messengers that trigger a cascade of signal transmission (Kinnamon, 1996; Gilbertson et al., 2000). As a consequence, signal is transmitted to the basal membrane, where the release of neurotransmitter occurs.

Different theories exist regarding the recognition of taste by TRC cells. According to one of them, the TRC cells respond to individual tastes and are innervated by "programmed" (tuned) to only one taste nerve fibers (labeled-line model). The other theory, called across-fiber pattern is described by two models. The first one assumes that a single TRC cell reacts to a series of flavors, and that the same afferent neurons transmit the information for more than one flavor. The second model assumes that the TRC cell receives individual tastes, but the same nerve fiber carries information for more than one flavor. Recent studies on mice have shown that various TRC cells recognize different tastes and that the activation of a single type of TRC cells is sufficient to create a particular taste, which supports the theory of labeled-line model (Chandrashekar et al., 2006).

It was observed that the TRC cells express only one type of GPCR receptors, such as bitter or sweet taste receptors, but never both simultaneously, and that a single afferent fiber can respond to two or more different flavors (such as sweet and bitter). Single sensory fibers respond to ATP released from the small number of neighboring TRC cells, which does not need to show the expression of the same type of taste receptor (Roper, 2007). TRC cells "programmed" to only one flavor, such as sweet, appeared to occur in close vicinity to TRC cells responsive to many tastes, such as sweet and bitter (presynaptic cells stimulated by ATP allocated by the TRC cells—sensitive to sweet or bitter substances). Afferent fibers respond largely to the ATP released

from the closest located TRC cells, but they can also respond to ATP liberated by a distant TRC cells, which may suggest that there are "sweet-best" or "bitter-best" sensory fibers, but not exclusively responsible for sweet or bitter substances (Roper, 2007).

GPCR RECEPTORS AND TASTE SENSING

GPCR receptors have been found to be involved in sweet, umami, bitter, and fat taste sensing (Amrein and Bray, 2003; Kim et al., 2004; Stewart et al., 2011). Substances causing taste of sweet and umami are recognized by the T1R family of receptors belonging to class C of GPCR receptors (Shi and Zhang, 2006). Substances causing a bitter taste are recognized by a large T2R family of taste receptors whose members belong mainly to class A or constitute separate subgroup within the GPCR receptors (Roper, 2007). It was shown that receptors responsible for the reception of fatty acids belong to GPCR receptors as well and those identified in human are: GPR120 located in all types of taste papillae, FFAR-3 (GPR41) and FFAR-2 (GPR43) found in circumvallate and foliate papillae, and FFAR-1 (GPR40) present only in the circumvallate papillae (Stewart et al., 2011).

SWEET AND UMAMI TASTE RECEPTORS

T1R family consists of three receptors: T1R1 and T1R2 (identified in 1999) and T1R3 (discovered in 2001) (Li, 2009). They are expressed in type II of the TRC cells and in the testes and small intestine (Ishimaru and Matsunami, 2009). It was shown that T1R receptors are responsible for recognizing sweet and umami tastes (Li et al., 2002). T1R2-T1R3 heterodimer functions as a sweet taste receptor, whereas T1R1-T1R3 heterodimer as a receptor for umami taste (Damak et al., 2003). Aside T1R1-T1R3 heterodimer, metabotropic glutamate receptors (mGlu) along with several ionotropic glutamate receptors were shown to be activated by compounds, which give rise to umami taste sensation (Kinnamon, 2009).

T1R1 receptor is expressed in fungiform papillae and the palate, T1R2 receptor almost exclusively in the circumvallate and foliate papillae (Hoon et al., 1999), and T1R3 receptor in all three types of taste papillae of the tongue (Nelson et al., 2001). Due to the pattern of the T1R receptor expression, TRC cells are divided into three types: co-expressing T1R1 and T1R3 (in fungiform papillae and the palate), co-expressing T1R2 and T1R3 (in circumvallate and foliate papillae, and palate), and expressing T1R3 only (in fungiform papillae and palate) (Bachamnov and Beauchamp, 2007). Although the T1R1 and T1R2 receptors are expressed in separate TRC cells, they are always co-expressed with T1R3 receptors (Shi and Zhang, 2006). Co-expression of T1R1 and T1R2 receptors has not been identified, indicating that the tastes of sweet and umami are caused by the activation of distinct cell types (Sugita, 2006).

Sweet Taste Signaling

A functional sweet taste receptor is a dimer of receptors T1R2 and T1R3 (Nelson et al., 2001; Ohkuri et al., 2009). In order to prove that the T1R2-T1R3 heterodimer functions as a sweet taste receptor, studies were conducted on knockout mice without the genes for T1R2 and/or T1R3. Mice lacking one of the genes (*T1R2* or *T1R3*) showed a blunted response to sugars, sweeteners, and D-amino acids, which confirms that the T1R2-T1R3 heterodimer functions as the main sweet taste receptor in vivo. However, tested mice showed very low, but measurable response to very high concentrations of natural sugars (>300 mM). This suggests that there may be additional sweet taste receptor activation pathway independent of T1R, or T1R2 and T1R3 can function alone as receptors for the natural sugars in the absence of their heteromeric partners. In mutants lacking both genes, i.e., *T1R2* and *T1R3*, a response to sweeteners has been completely eliminated (Zhao et al., 2003).

In the mouse, *Sac* gene locus located on chromosome 4 is a major determinant of the differences between sensitive and insensitive strains of mice in their response to sucrose, saccharin, and other sweeteners. During search for the mouse homolog of the gene, syntenic *Sac* fragment of approximately one million base pairs in the human genome was analyzed and as a result the gene for T1R3 receptor was identified (Lewcock and Reed, 2001; Max et al., 2001).

T1R2-T1R3 heterodimer recognizes a wide variety of sweet substances ranging from natural sugars (sucrose, fructose, glucose, maltose), sweet amino acids (D-tryptophan, D-phenylalanine, D-serine), and the sweeteners (aspartame, cyclamate, saccharin) to the sweet protein (monellin, brazzein, thaumatin) (Jiang et al., 2005). Humans and mice have different ability to identify sweeteners and sweet proteins. For example, mice do not respond to aspartame or monellin. However, the introduction of human *T1R2* gene into mouse genome changed their taste preferences for similar to those seen in humans (Zhao et al., 2003).

Functional studies of T1R have shown that in humans cysteine-rich domain of T1R3 is required for the response to the sweet protein, such as brazzein and monellin (Bachmanov and Beauchamp, 2007), and the transmembrane domains of T1R3 are responsible for interaction with cyclamate and lactisole (inverse agonist, so-called anti-sugar; Palmer, 2007). Extracellular N-terminal domain of T1R2 receptor is involved in the recognition of aspartame, neotame, D-tryptophan, and sucrose. T1R2 transmembrane domains, in turn, are responsible for the coupling of G proteins with T1R2-T1R3 heterodimers (Nie et al., 2005; Bachmanov and Beauchamp, 2007).

Biochemical and electrophysiological studies indicated that the TRC cell response to the sweeteners is committed to Gs and Gq proteins. It was stated that the gustducin may also participate in signal transduction of sweet taste since mice lacking this protein showed a reduced response to sweet substances, such as sucrose, proline, tryptophan, or sweeteners, including saccharine (Margolskee, 2002). Gustducin belongs to the class

$G_{i/o}$ of G proteins and shows significant similarity to other G protein, i.e., transducin (about 80% amino acid sequence identity), which is present in the retina of vertebrates (Lerea et al., 1986; Spielmann, 1998; Palmer, 2007). Gustducin is expressed in approximately 25–30% of the TRC cells of the palate and in all types of taste papillae (Gilbertson et al., 2000). It was found that gustducin is responsible for the activation of phosphodiesterase (PDE), which hydrolyses cyclic adenosine monophosphate (cAMP) to AMP (Clapp et al., 2001).

Signal transduction of sweet taste occurs via two distinct signaling pathways. The first one involves cAMP and the second one involves inositol 1,4,5-trisphosphate (IP3). It was shown that sugars cause an increase of cAMP, while sweeteners stimulate the production of IP3 (Varkevisser and Kinnamon, 2000).

Briefly, sucrose or other sugar binding to the T1R receptor through Gs protein activates adenylate cyclase (AC) that converts ATP to cAMP. cAMP can directly cause the influx of cations through cyclic nucleotide-dependent ion channels, or activate protein kinase A (PKA) that through phosphorylation of K^+ channels present in the basal membrane causes them to shut down. This leads to TRC cell depolarization resulting in the influx of calcium ions through voltage-gated calcium channels and neurotransmitter release (Margolskee, 2002). Expression of cyclic nucleotide gated ion channel was noted in TRC cells, which may also contribute to membrane depolarization and influx of calcium as a result of elevated levels of cAMP (Lindemann, 2001).

In turn, sweetener binding to the T1R receptor activates Gq proteins. Dissociated subunits Gqα or Gq$\beta\gamma$ stimulate phospholipase C $\beta 2$ (PLC $\beta 2$) that hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to diacylglycerol (DAG) and IP3 (Margolskee, 2002). Elevated level of IP3 causes the release of calcium ions from intracellular compartments leading to TRC cell membrane depolarization and release of neurotransmitter (Ohkuri et al., 2009).

Different routes of responses to sucrose and non-sugar sweeteners were established in rat model. It was observed that cAMP level in rat cells after stimulation with non-sugar sweeteners is significantly lower than after stimulation with sucrose, while the level of IP3 is higher in response to non-sugar sweeteners and lower in response to sucrose. In response to both, sucrose and non-sugar sweeteners, TRC cells showed elevated level of calcium. However, only in the case of sweetener increased concentration of Ca^{2+} appeared to be independent of extracellular calcium because it was caused by the release of calcium from intracellular compartments (Lindemann, 1996).

Umami Taste Signaling

The taste of umami is primarily caused by L-glutamate present in the diet mainly in the form of monosodium glutamate (MSG; Roper, 2007). L-glutamate occurs naturally in many foods, mainly meat, cheese, seafood, fish, milk, tomatoes, and other vegetables (Fuke and Ueda, 1996; McCabe and Rolls, 2007). In 1908, Kikunae Ikeda isolated L-glutamate from konbu/kombu (dried kelp of *Fucus vesiculosus*) and described its taste as unique, very different from other flavors. He has called this taste "umami." The Japanese word "umai" means "delicious" (Kinnamon, 2009). The taste of umami is caused also by other amino acids (e.g., L-aspartate), some dipeptides, tripeptides, oligopeptides, and synthetic ingredients similar to glutamate, such as L-2-amino-4-phosphonobutanoic acid (L-AP4) and some organic acids (Figure 2; Roper, 2007). The umami taste is reinforced by the presence of 5'-ribonucleotides: inosine 5'-monophosphate (IMP) and guanosine-5'-monophosphate (GMP; Chaudhari et al., 2000), which themselves are also able to cause this taste (Kinnamon, 2009).

So far, in the TRC cell several receptors capable of L-glutamate binding were identified, e.g., T1R1-T1R3 heterodimer, specific for taste isoforms of metabotropic glutamate receptors: mGlu1, mGlu2, mGlu3, and mGlu4, and several ionotropic receptors including N-methyl-D-aspartate (NMDA) and kainate receptors (KARs). The role of these receptors in umami taste signal transduction is not clear since glutamate also functions as a neurotransmitter. It is believed that umami taste receptors that respond to the glutamate present in food are expressed in the apical membrane of the TRC cell while NMDA and KARs are located in the basal membrane of TRC cells, where they are likely to respond to the glutamate acting as a neurotransmitter (Kinnamon, 2009).

A shortened form of mGlu4 (called taste-mGlu4) commonly occurring in the central nervous system was identified in rat taste buds (Chaudhari et al., 2000; Lindemann et al., 2002). This form of mGlu4 has approximately 50% shorter N-terminal extracellular domain in which the binding site of L-glutamate is

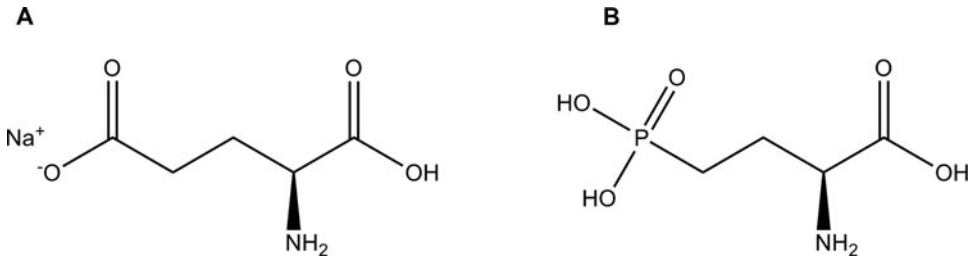

Figure 2 Chemical compounds responsible for umami taste sensation; A – monosodium glutamate (MSG), B - 2-amino-4-phosphonobutanoic acid (L-AP4).

localized, and thus is characterized by reduced affinity to the ligand. However, it was shown that taste-mGlu4 is expressed in the TRC cells and binds L-AP4 that gives the taste similar to MSG in rats and humans (Chaudhari et al., 2000). Ligand binding to taste-mGlu4 leads to activation of PDE and decreased cAMP level. It was found that the presence of 5'-ribonucleotides reinforces mGlu4 activation by taste ligands. Further steps in signal transduction are still unclear, but it is believed that low level of cAMP can unlock the ion channels inhibited by cyclic nucleotides, which can lead to an increase of intracellular calcium and neurotransmitter release (Gilbertson et al., 2000).

In 2003, T1R1-T1R3 heterodimer was identified as a new functional receptor for umami taste, which showed a response to L-glutamate and other amino acids. The addition of IMP in low concentrations enhances the receptor response to these ligands (Roper, 2007). In rodents, T1R1-T1R3 heterodimer recognizes all L-amino acids typical for proteins, while in humans responds exclusively to L-glutamate and L-aspartate (Ishimaru, 2009). In cells located in fungiform papillae and taste palate, T1R1-T1R3 heterodimer is co-expressed with α-gustducin, while in circumvallate papillae and foliate papillae with $G_{\alpha i2}$ protein (Kinnamon, 2009). In the taste buds, the expression of α-transducin was also noticed but at a much lower level than the α-gustducin. It is suggested that α-transducin and α-gustducin are involved in umami taste signal transduction because mice lacking the gene for one of these proteins showed a reduced response to this taste (He et al., 2004). In the taste palate fungiform papillae, α-gustducin and α-transducin activate PDE that reduces cAMP level. In the circumvallate and foliate papillae, a decrease in cAMP level is observed, however, probably due to AC inhibition rather than PDE activation (Kinnamon, 2009).

Zhao et al. (2003) found that mice lacking T1R1 or T1R3 receptors showed no response to MSG and other amino acids. In turn, Damak et al. (2003) observed that mice lacking T1R3 showed a reduced but still measurable response to MSG, which may indicate that umami taste is recognized not only by the T1R1-T1R3 heterodimers, but also by other receptors, such as a shortened version of mGlu4, identified in 2005 taste-mGlu1 (which is a shortened form of mGlu1) or other yet undiscovered receptors (Roper, 2007). As a result of ligand binding to the T1R1-T1R3 heterodimer, released $G\beta\gamma$ subunits stimulate PLC $\beta 2$, which hydrolyzes PIP2 to DAG and IP3 (Kinnamon, 2009). IP3 activates inositol-1,4,5-trisphosphate receptors type 3 (IP3R3) and in consequence the release of calcium ions from intracellular compartments (Clapp et al., 2001). Calcium ions activate TRPM5 channel (transient receptor potential (TRP) cation channel subfamily M member 5/fifth member of melastin-related TRP channel subfamily), influx of sodium ions, cell membrane depolarization, and finally release of ATP, which activates ionotropic purinergic receptors located in sensory fibers (Peréz et al., 2002; Sugita, 2006). Mice devoid of TRPM5, PLC $\beta 2$, and IP3R3 showed a reduced response to substances that cause taste of umami, which confirms their role in umami taste signaling (Kinnamon, 2009).

BITTER TASTE RECEPTORS AND SIGNALING

Bitter substances are recognized by T2R receptors family (also known as TRB or Tas2R; Shi and Zhang, 2009; Temussi, 2009). In humans, 25 bitter taste receptors have been identified (Upadhyaya et al., 2010), while in the mouse 34 T2R family members (Ishimaru, 2009). T2R receptors are expressed in TRC cells of tongue and palate (Chandrashekar et al., 2000). It was shown that T2R receptors are also present in the gastrointestinal tract where they are likely to play a role in the emetic reflex, but their exact functions remain unknown (Wu et al., 2002).

In contrast to the T1R, T2R receptors have short extracellular N-terminal domain (Maehashi and Huang, 2009). It is assumed that, like T1R these receptors are capable to form dimers, but as yet no evidence supporting this hypothesis has been known (Fotiadis et al., 2006; Roper, 2007). T2R receptors are characterized by highly evolutionarily conserved motifs in cytoplasmatic loops and transmembrane domains, and variability in extracellular region that is required for the recognition of structurally different ligands (Maehashi and Huang, 2009). The attachment site of the flavors corresponds to the first and the second extracellular loop and transmembrane domains TM 1, 2, 3, and 7 (Upadhyaya et al., 2010).

T2R receptors are encoded by a family of genes in which the vast majority are pseudogenes (pseudogenes in mice amount 17%, while in humans 40% of the T2R genes). Genes for T2R are characterized by the lack of introns, in contrast to the T1R genes (Andres-Barquin and Conte, 2004; Parry et al., 2004; Shi and Zhang, 2006). In humans, T2R genes are located on chromosomes 5, 7, and 12, but most of them occur in two clusters of 10 genes on chromosome 7 and 20 genes on chromosome 12 (Andres-Barquin and Conte, 2004). In mice, genes responsible for sensitivity to bitter substances are located on chromosome 6 (Montmayeur and Matsunami, 2002).

Studies on rodent cells have shown that T2R receptors are expressed in about 15% of the circumvallate and foliate papillae, and only in about 2% of fungiform papillae taste buds (Montmayeur and Matsunami, 2002). Despite the fact that in the same taste buds TRC cells expressing either T1R or T2R receptors can be found, the T1R and T2R receptors are not co-expressed in the same TRC cell (Amrein and Bray, 2003; Sugita, 2006). Most of the T2R receptors are co-expressed in the same subtype of TRC cells, which allows recognition of a huge variety of bitter substances, but without the ability to distinguish them (Adler et al., 2000; Sugita, 2006).

Initially, it was thought that bitter substances stimulate all of the cells expressing T2R receptors. However, further studies have shown that individual cell shows a response to one or more, but not all bitter ligands. These conflicting results are explained by team of Behrens, who stated that human TRC cells sensitive to bitter substances exhibit expression of only certain subclasses of T2R, and not the entire family of the receptors (Behrens et al., 2006).

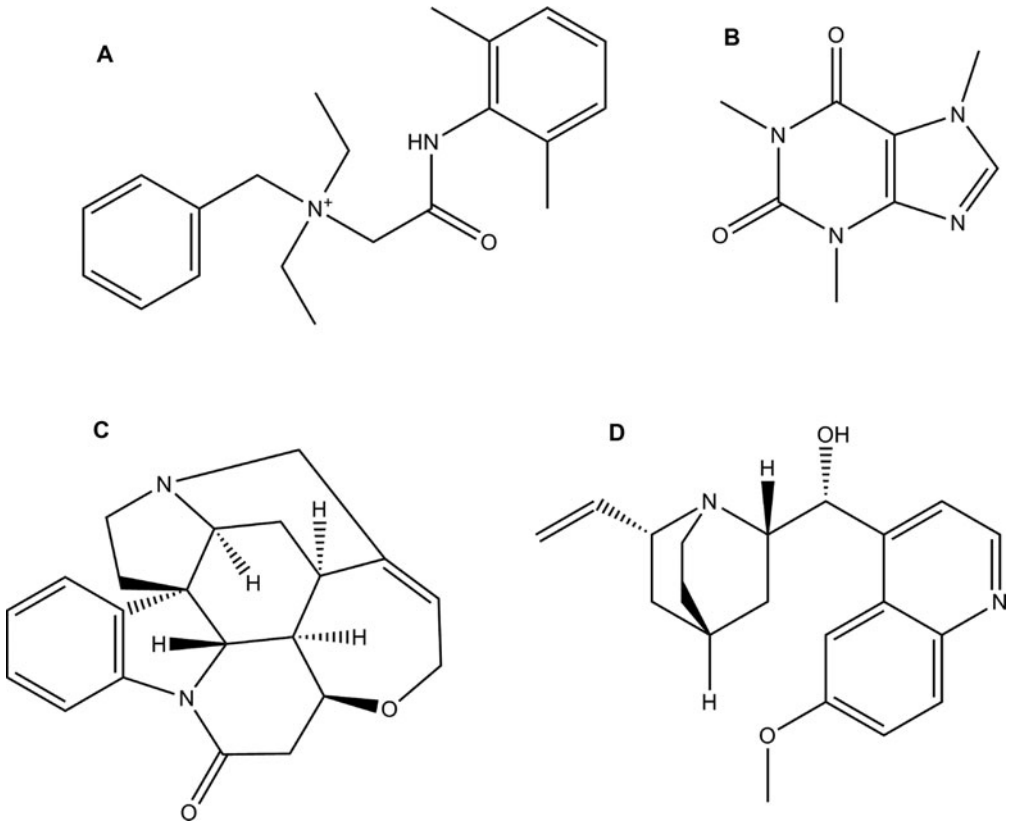

Figure 3 Selected chemical compounds responsible for bitter taste sensation; A – denatonium, B – caffeine, C – strychnine, D – qunine.

Ligands for T2R receptors are amides, such as denatonium (phenylmethyl-[2-[(2,6-dimethylphenyl)amino]-2-oxoethyl]-diethylammonium benzoate), alkaloids, such as strychnine, caffeine, and quinine, some amino acids, urea, fatty acids, phenols, amines, and even potassium, magnesium, and calcium salts (Figure 3; Roper, 2007). Due to the substrate specificity, T2R receptors can be activated by many different bitter substances or recognized by a few bitter ligands only (Table 1; Upadhyaya et al., 2010). The family of T2R receptors includes many orphan receptors, for which ligands have not yet been identified (Temussi, 2009).

Table 1 Selected ligands for human (h) bitter taste GPCR receptors from T2R family (Pronin et al., 2004; Maehashi and Huang, 2009; modified)

Receptor	Ligands
hT2R1	Bitter peptides, e.g., produced during food processing (fermentation) or ageing
hT2R4	6-n-propyl-2-thiouracil (PROP) denatonium
hT2R7	Strychnine, quinacrine, chloroquine, papaverine
hT2R10	Strychnine
hT2R16	β-glucopyranosides, e.g., salicin
hT2R38	6-n-propyl-2-thiouracil (PROP)
hT2R43	6-nitrosaccharin, aristolochic acid, denatonium
hT2R44	6-nitrosaccharin, denatonium
hT2R46	Strychnine
hT2R47	6-nitrosaccharin, aristolochic acid, denatonium
hT2R61	6-nitrosaccharin

It was found that α-gustducin co-expressed with T2R receptors in the TRC cells is involved in the bitter taste signal transduction (Ishimaru, 2009; Shi and Zhang, 2009). Mice lacking the gene for α-gustducin showed a reduced response to inducers of sweet, umami, and bitter tastes, which confirms its role in these taste signal transductions (Sugita, 2006). Bitter substance binding to its cognate receptor leads to activation of gustducin, which α subunit causes a decrease of cAMP level due to activation of PDE. The $\beta\gamma$ subunits lead to increase of IP3 level and activation of the IP3R3 receptor, resulting in the release of calcium ions from intracellular compartments (Andres-Barquin and Conte, 2004). Although, α-gustducin/PDE/cAMP pathway is not fully understood, it is suggested that low level of cAMP may affect the protein kinase, which in turn may regulate ion channel activity of TRC cells. The decrease in cAMP can also directly modulate the activity of ion channels dependent on cyclic nucleotides, which can lead to increased level of intracellular calcium ions and release of neurotransmitter (Margolskee, 2002).

FAT TASTE RECEPTORS AND SIGNALING

The existence of separate transmission of the taste of fat remains a topic of debate. Previous studies revealed the presence of receptors on the surface of the TRC cells for which natural ligands are FFAs (Matsumura et al., 2007; Cartoni et al., 2010; Stewart et al., 2011). There is growing evidence on human and animal ability to detect FFAs' presence in food. Recently,

Stewart et al. (2010) reported that humans are capable of detecting FFAs in oral cavity. The occurrence of dedicated receptors, expression of which have been confirmed in TRC cells, as well as identification of signal transduction pathways and physiological studies prove that the taste of fat should be included to the existing canon of five fundamental flavors (Mattes, 2009). At least five GPCR receptors that bind FFAs have been identified in rodent taste cells. They are FFAR-1 (GPR40), FFAR-3 (GPR41), FFAR-2 (GPR43), GPR84, and GPR120 (Mattes, 2011). Out of these receptors, FFAR-1 and GPR120 are best known and studied. FFAR-1 receptor is fairly distributed in many tissues, including pancreatic islets (2–100-fold upregulated than in total pancreas), brain (substantia nigra and medulla oblongata region), and monocytes, with minor expression in heart, liver, and skeletal muscle (Briscoe et al., 2003; Kotarsky et al., 2003). Lately, FFAR-1 expression was identified in TRC cells of types I and II (Matsumura et al., 2007; Cartoni et al., 2010). The exact mechanism of FFAR-1 receptor physiological relevance remains to be revealed. However, dose-dependent upregulation of Ca^{2+} concentration in the cell in response to FFA stimulation was reported (Gromada, 2006). Moreover, similar to other taste receptors, a reduction in plasma membrane current through voltage-gated K^+ channels and increased activity of PKA were observed (Feng et al., 2006).

Expression of the GPR120 receptor has been identified in lung, intestinal, and adipose tissues, as well as in the circumvallate papillae (Gotoh et al., 2007; Matsumura et al., 2007; Cartoni et al., 2010). GPR120 is generally connected with FFA-induced secretion of glucagon-like peptide-1 and cholecystokinin (Hirasawa et al., 2005; Tanaka et al., 2008).

DOWNSTREAM TASTE SIGNALING EFFECTORS

Binding of ligands for the taste of sweet, umami, and bitter to T1R and T2R receptors located in the apical membrane of type II TRC cells leads to the dissociation of heterotrimeric G proteins and activation of two major signal transduction pathways. The first one relay on the cAMP. It is suggested that α subunit of G proteins may cause both, increase of cAMP level by AC activation and decrease of cAMP level by PDE activation. Elevated level of cAMP activates PKA kinase, which prosphorylates potassium channel causing its closing and TRC cells membrane depolarization. Stimulation of voltage-gated calcium channels and influx of calcium ions, ultimately, leads to the release of neurotransmitter (Roper, 2007). It was shown that stimulation of taste receptor may also lead to change of cGMP level through activation of α subunits of G proteins (Sugita, 2006). Sucrose binding causes an initial increase of cGMP level, which seems to occur earlier and faster than the transient changes in the level of cAMP. It is suggested that changes in cAMP level occur later and may be involved in long-term processes such as adaptation (Roper, 2007).

The second taste signaling pathway occurs with the participation of PLC $\beta 2$, which is activated by the $\beta\gamma$ subunits of G proteins (Roper, 2007). PLC $\beta 2$ hydrolyzes PIP2 to DAG and IP3. Binding of IP3 to the IP3R3 receptor leads to the release of calcium ions from intracellular compartments (Ishimaru and Matsunami, 2009). Mice lacking the gene for PLC $\beta 2$ and IP3R3 showed no response to substances causing sweet, bitter, and umami tastes, confirming the involvement of these proteins in signal transduction of these tastes (Ishimaru, 2009). The rapid increase in intracellular calcium level activates TRPM5 channels leading to an influx of sodium ions and membrane depolarization (Sugita, 2006). Finally, type II TRC cells release ATP that acts on the receptors present on the surface of purinergic type III TRC cells and induces the release of secretin into the synapse between type III TRC cells and the sensory neurons. It is suggested that ATP can also directly affect the afferent fibers (Figure 4; Palmer, 2007).

It was found that over half of all TRC cells express the receptor ion channel TRPM5 (Mombaerts, 2004). This channel permeable only to monovalent cations, gated by intracellular calcium ion concentration is co-expressed with PLC $\beta 2$ and T1R and T2R receptors in TRC cells (Zhang et al., 2003; Fleig and Penner, 2004; Liman, 2007). TRPM5 has short N-terminal, six transmembrane, and large C-terminal domains (Palmer, 2007). TRPM5 does not display constitutive activity, but can be activated by calcium ions released from intracellular compartments as a result of receptor stimulation (Hofmann et al., 2003; Minke, 2006). It was shown that temperature increase of about 15–35°C stimulates the TRPM5 in the presence of cytosolic calcium ions (Sugita, 2006). TRPM5 activity is also regulated by the voltage and pH (Liman, 2007).

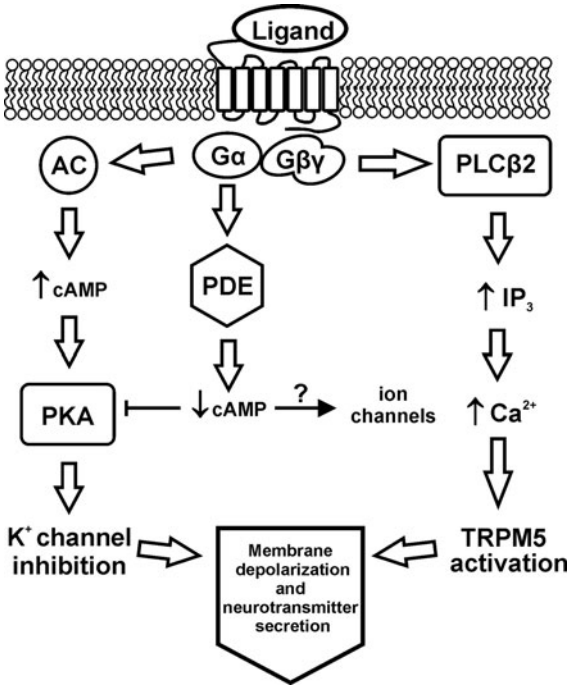

Figure 4 Schematic representation of taste signal transduction in TRC cells; AC – adenylyl cyclase, cAMP – cyclic adenosine monophosphate, Gα, $\beta\gamma$ – subunits of G protein trimeric complex, IP3 – inositol 1,4,5-trisphosphate, PDE – phosphodiesterase, PKA – protein kinase A, PLC $\beta 2$ – phospholipase C $\beta 2$, TRPM5 - transient receptor potential cation channel subfamily M member 5/fifth member of melastin related TRP channel subfamily.

Recent reports suggest that TRPM5 is involved in signal transduction of sweet, umami, and bitter tastes. However, mice lacking the gene for TRPM5 showed two phenotypes of response to sweet, umami, and bitter substances. The first is manifested by the total lack of response to these substances, while the second by a reduced but still measurable response to taste ligands. These discrepancies between the results were probably due to differences in the construction of target vectors used for testing (Ishimaru and Matsunami, 2009).

PERSPECTIVES

In this review we have summarized the importance of GPCR receptors for taste sense physiology. These molecules have become focal point of pharmaceutical industry as targets for pharmacological intervention, all together bringing more than 16 billion dollars of revenue (Schlyer and Horuk, 2006). Ongoing and future investigations open new possibilities also for food industry as GPCR receptors seem to be ideal molecules for both natural and synthetic food additives, e.g., taste enhancers that allow lower salt and sugar usage in food.

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