Biological Characteristics and Signal Transduction Mechanisms of Bitter Taste Receptors, and Effects of Bitter Tastants and Inhibitors: Postprint

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Abstract

Bitter taste receptors (TAS2Rs) are G protein-coupled receptors (GPCRs) encoded by a gene family comprising 30 genes. Bitterness enables animals to avoid toxic and harmful substances; when animals taste bitter compounds, taste receptor cells in the taste buds of the tongue are stimulated to express TAS2Rs, thereby triggering a cascade of downstream signal transduction reactions that ultimately integrate and transmit information to the brain via the chorda tympani and glossopharyngeal nerves, generating an aversive sensation that prompts animals to reject intake of these bitter substances. This article provides a brief review of the biological characteristics of TAS2Rs, their signal transduction mechanisms, and the effects of bitter agonists and bitter inhibitors on bitter taste receptors.

Full Text

Bitter Taste Receptors: Biological Characteristics, Signal Transduction Mechanism, and Effects of Bitter Agents and Bitter Taste Inhibitors

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Abstract: Bitter taste receptors (TAS2Rs) are G-protein-coupled receptors (GPCRs) encoded by a gene family comprising 30 genes. Bitterness enables animals to avoid toxic and harmful substances. When animals encounter bitter

compounds, taste receptor cells in the tongue' s taste buds express TAS2Rs, triggering a cascade of downstream signal transduction events. This information is ultimately transmitted to the brain via the chorda tympani and glossopharyngeal nerves, generating an aversive sensation that causes animals to reject these bitter substances. This article provides a brief review of the biological characteristics of TAS2Rs, their signal transduction mechanisms, and the effects of bitter agents and bitter taste inhibitors on bitter taste receptors.

Keywords: bitter taste receptor; biological characteristic; signal transduction mechanism; bitter agent; bitter taste inhibitor

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Taste refers to the sensation produced by the chemical sensory system of taste organs in the animal oral cavity in response to food stimuli. Mammals can generally perceive five basic tastes: sour, sweet, bitter, salty, and umami. Among these, bitterness plays a crucial role for animals. Many toxic and harmful substances possess bitter qualities, and when animals encounter bitter-tasting feed, they develop a strong aversion—an important defense mechanism against the ingestion of toxins [1]. Bitter taste receptors (TAS2Rs) are seven-transmembrane G-protein-coupled receptors (GPCRs), and bitter taste transduction in taste receptor cells is primarily mediated by G proteins and GPCRs. This article provides a brief review of the biological characteristics of TAS2Rs, their signal transduction mechanisms, and the effects of bitter agents and bitter taste inhibitors on bitter taste receptors.

1 Biological Characteristics of TAS2Rs

TAS2Rs are GPCRs with a seven-transmembrane helical structure formed from a single polypeptide chain [Figure 1: see original paper] [2], containing three intracellular loops and three extracellular loops. In TAS2Rs, the transmembrane regions exhibit the highest degree of conservation, followed by the intracellular regions. The three intracellular loops are highly conserved and serve as G protein-coupling domains, while the extracellular regions show the greatest variation. The short N-terminal extracellular domain displays pronounced polymorphism and can bind various bitter substances, suggesting it functions as the ligand-binding region. The coding sequences of bitter receptor genes in mammals are composed of a single exon, though their lengths vary slightly among species. For example, the gene is 912 bp in dogs and mice, 906 bp in horses, 915 bp in black bears, and reaches 1,189 bp in pigs [3]. Between 30% and 70% of amino acid sequences are conserved among TAS2R family members. Additionally, Wei Chengxiao [4] demonstrated that the structure of porcine bitter receptor genes is essentially identical to that of humans, mice, and other mammals.

1.2 Distribution and Gene Expression Regulation

TAS2Rs are expressed not only in tongue taste buds but also in various other animal tissues, where they exhibit distinct biological functions. Studies have shown that some TAS2Rs and their homologous α-gustducin are expressed in human airway smooth muscle [5]. Shah et al. [6] confirmed that TAS2Rs expressed in motile cilia of airway epithelial cells can increase ciliary beat frequency, which may represent a mechanical defense mechanism protecting the respiratory tract from toxic gases. Wu et al. [7] also identified TAS2R expression with known ligands in gastrointestinal tissues and cells. Their results revealed that TAS2R108 and TAS2R138 were detected in the gastric antrum, gastric fundus, duodenum, and tongue, but not in the liver, heart, or kidney. Additionally, the rodent-specific TAS2R134 showed similar expression patterns in the stomach, duodenum, and tongue. These findings demonstrate that TAS2R-encoding genes are expressed in the gastrointestinal mucosa of rodents. Furthermore, Singh et al. [8] detected transcripts of TAS2R104, TAS2R107, and TAS2R138 in cultured C6 glioma cells, brainstem, cerebellum, cortex, and nucleus accumbens of mouse brain using real-time fluorescent quantitative PCR (RT-PCR), and also identified TAS2R104 in primary neurons.

1.3 Chromosomal Localization

Rozengurt [9] conducted homology-based bioinformatic screening of bitter taste receptor genomes in rodents to identify family-related sequences. The results showed that bitter receptor genes are unevenly distributed across three chromosomes: two chromosomes each contain a single gene, while the third chromosome harbors a cluster of remaining TAS2R functional genes and pseudogenes. Based on homology with the mouse genome and their positions on chromosomes 15, 2, and 6, mouse bitter receptor genes were divided into three subgroups. Similarly, 36 bitter receptor genes in rats are distributed across chromosomes 2, 3, and 4. Human bitter receptor genomes contain 25 members [10], with one gene on chromosome 5, an extended cluster of nine genes on chromosome 7, and the remaining 15 genes in a dense cluster on chromosome 12 [11]. The phylogenetic relationships of functional TAS2R genes between humans and mice are illustrated in Figure 2 [Figure 2: see original paper] [12]. Additionally, Li et al. [13] found that pigs possess 23 bitter receptor genes, cattle have 34, dogs have 15, while chickens have only three. The chromosomal distribution of bitter receptor genes is not random; their concentration in specific chromosomal regions likely results from gene duplication events.

2 Signal Transduction Mechanisms of TAS2Rs

Given the diverse chemical structures of bitter substances, multiple signal transduction pathways likely exist. Gustducin plays a crucial role in TAS2R responses to bitter compounds. TAS2R activation promotes rapid changes in second messengers through a signaling pathway involving the G protein gustducin [9]. This heterotrimeric G protein mediates two distinct signal transduction

pathways via its α-subunit and $\beta\gamma$-subunit, as shown in Figure 3 [Figure 3: see original paper] [14]. In one pathway, bitter compounds such as cycloheximide bind TAS2Rs, activating the G protein α-subunit, which reduces intracellular cyclic adenosine monophosphate (cAMP) levels through phosphodiesterase activation. This decrease in cAMP relieves inhibition of cAMP-gated channels, triggering release of Ca^{2+} from intracellular stores, increasing Ca^{2+} concentration and causing membrane depolarization. In the alternative pathway, some bitter compounds bind TAS2Rs and activate the $\beta\gamma$-subunit of gustducin, which stimulates phospholipase C$ 2$(PLC\ 2)tosynthesizeinositol1,4,5 - triphosphate$(IP3), leadingtoCa\hat{}\{2+\}$ release from intracellular stores, depolarization of bitter receptor cells, and neurotransmitter release [15]. Studies have demonstrated that gustducin functions as a signal cascade component in taste cell transduction [16]. Additionally, bitter substances can modulate gating of voltage-sensitive ion channels and mediate Ca^{2+} influx into cells. Recent research indicates that a transient receptor potential channel, transient receptor potential cation channel subfamily M member 5 (TRPM5), is essential for bitter taste signaling in lingual epithelium. Bitter compounds in the lingual epithelium induce increased intracellular Ca^{2+} concentration and trigger ATP release, which activates purinergic receptors on nerve fibers, enabling these receptors to control the encoding and integration of taste information in sensory centers [17].

Some mice lacking gustducin genes can still perceive bitter substances, suggesting the existence of G protein-independent bitter compounds that can directly interact with TAS2Rs to open ion channels. Additionally, substances such as quinine can close K^+ channels, leading to TAS2R depolarization. Recent pharmacological studies have shown that denatonium benzoate (DB) can reduce cAMP levels while increasing PLC$ 2activity, ultimatelyelevatingintracellularCa\hat{}\{2+\}$ concentration and promoting hormone release [18]. This indicates that a single substance may utilize multiple transduction pathways that are not completely independent but rather interconnected.

3 Effects of Bitter Agents on TAS2Rs

3.1 Tannins

Tannins, which possess astringent and bitter tastes, are among the most common antinutritional factors found in numerous feeds and forages. These water-soluble compounds are classified into two categories: condensed tannins and hydrolyzable tannins. As antinutritional factors, condensed tannins exert dose-dependent effects on animals, particularly ruminants. Low concentrations may provide certain nutritional benefits, while high concentrations cause antinutritional effects. Research has shown that condensed tannins inhibit feed intake and complex with dietary proteins or other components, digestive enzymes, and endogenous protein losses in ruminants, while also exerting toxicity in other tissues [19]. Therefore, mitigating the antinutritional effects of tannins is crucial.

Hydrolyzable tannins are toxic compounds that affect duodenal morphological characteristics by increasing villus height, mucosal thickness, and villus circumference, though other small intestinal regions remain unaffected [20]. Tannins reduce cell mitosis and apoptosis but have no adverse effects on the liver.

Different tannin types bind distinct TAS2Rs. Epicatechin, a condensed tannin precursor, binds TAS2R4, TAS2R5, and TAS2R39. The hydrolyzable tannin pentagalloylglucose (PGG) interacts with TAS2R5 and TAS2R39, while procyanidin trimers, a type of condensed tannin, bind only TAS2R5. Tannins represent the first natural agonists of TAS2R5 among bitter substances, capable of inducing substantial TAS2R5 mRNA expression [21].

3.2 Phenylthiocarbamide (PTC)

PTC is a toxic white crystalline substance with a pungent odor that tastes bitter due to its thiocyanate (NC=O) molecular structure. Mani et al. [22] conducted a trial with 16 pigs divided equally between a group fed a diet containing 1 mmol PTC and a control group. No difference in feed consumption was observed between the two groups, indicating that PTC maintained relatively good palatability for pigs, though gastric volume was significantly higher in the PTC-fed group. Comparison of gastrointestinal chyme retention revealed more pronounced retention in the PTC-fed group, with gastric volume decreasing by 30% at 45 minutes post-feeding. These data demonstrate that PTC bitter compounds can slow gastric emptying. Measurements of nutrient transport in the anterior jejunum showed that glucose, lysine, and glutamine transport increased by 250% in the PTC-fed group.

Research indicates that PTC binds TAS2R38, increasing TAS2R38 mRNA expression and transmitting bitter signals to the cerebral cortex. This occurs because the four haplotype genes of TAS2R38—TAS2R38PAV, TAS2R38AVI, TAS2R38AAI, and TAS2R38PVV—exhibit substantially different responses to PTC. Tan et al. [23] predicted binding sites by docking PTC with the four TAS2R38 haplotypes, finding that PTC forms hydrogen bonds with the first three haplotypes at residue 262 but not with the TAS2R38PVV haplotype. This suggests that hydrogen bond interactions between transmembrane helices 3-6 of TAS2R38 can activate receptor signaling upon ligand binding, with the hydrogen bond formed by PTC at residue 262 participating in bitter taste formation.

3.3 Soy Isoflavones

Soy isoflavones are widely present in soybean-containing energy feeds and impart a bitter taste that causes animals to reject such feeds. Structurally similar to mammalian estrogen, soy isoflavones can act as estrogen agonists or antagonists. Roland et al. [24] found that soy isoflavones bind TAS2R14 and TAS2R39 through interactions involving two hydrogen bond donor sites, one hydrogen bond acceptor site, and two aromatic ring structures, leading to receptor activation and bitter taste generation.

Different bitter substances can bind one or multiple TAS2Rs and increase TAS2R mRNA expression. Various bitter compounds exist in feed, such as alkaloids in legume-containing feeds that possess antioxidant properties, and bitter glucosinolates in cruciferous plants like cabbage, which can cause poisoning in dairy cows when fed in excess [25]. Low concentrations of these substances may provide health benefits, while high concentrations can cause toxicity or even death. Therefore, inhibiting bitter taste generation in feed is particularly important.

4 Bitter Taste Inhibitors

4.1 Amino Acids and Their Derivatives

Amino acid derivatives and peptides are known bitter masking agents, though their mechanisms—whether acting at the receptor level or on intracellular taste signaling cascade components—remain poorly understood. Previous studies have shown that L-ornithyl-β-alanine (OA) can mask bitterness in potassium salts, while γ-aminobutyric acid (GABA) can mask the bitterness of quinine, caffeine, cocoa, and chocolate [26]. Pydi et al. [27] used molecular modeling-guided mutagenesis to first predict quinine binding sites in TAS2R4, then docked 75 amino acid derivatives with the TAS2R4 ligand-binding site. Approximately 19 amino acid derivatives, including GABA and OA, showed binding affinities within the predicted range. N(α)-benzyloxycarbonyl-L-tryptophan methyl ester and N,N-bis(carboxymethyl)-L-lysine (BCLM) exhibited the highest binding affinities. These two compounds, along with GABA and OA, were selected for specific competitive analysis. Competitive Ca^{2+} mobilization assays revealed that only GABA and BCLM displayed antagonist activity, while the other two could not inhibit TAS2R4.

Constitutively activating mutations in GPCRs serve as pharmacological tools to classify ligands as neutral antagonists or inverse agonists [28-29]. Neutral antagonists are compounds that do not affect basal receptor activity, whereas inverse agonists reduce receptor activity. Studies have shown that BCLM can reduce receptor activity and inhibit TAS2R mRNA expression, while GABA has no such effect [27]. This makes GABA the first endogenous bitter taste receptor antagonist among amino acid derivatives and peptide compounds, widely applied as a safe, low-toxicity feed additive to eliminate bitterness in animal feed. BCLM represents the first antagonist with inverse agonist properties for bitter taste masking.

4.2 Adenosine Monophosphate (AMP) and Its Analogs

AMP and its analogs can mask bitterness in animal feed. These bitter inhibitors include 5' -AMP, 5' -adenosine diphosphate, 5' -thymidine monophosphate, 5' -succinyl adenosine, 5' -ATP, and 3' -AMP [30]. AMP shows the strongest bitter inhibitory effect and was the first adenosine compound discovered to suppress bitterness, exhibiting varying degrees of inhibition against multiple

bitter compounds including DB, nicotine, and caffeine.

Margolskee et al. [31] found that AMP can attach to bitter taste receptor cells, inhibiting TAS2R mRNA expression and reducing bitter perception by decreasing taste nerve transduction levels. As a bitter control agent, flavor enhancer, and taste corrector, AMP can be applied in feed to eliminate bitterness and improve animal welfare.

4.3 Phosphatidic Acid (PA) and Its Complexes

Most bitter compounds contain hydrophobic groups, and phospholipids—being typical hydrophobic substances—can shield TAS2Rs and competitively inhibit bitter taste. Although lecithin and cephalin have long been used abroad to suppress bitterness with limited success, studies show that among phospholipid compounds, PA exhibits the strongest bitter inhibitory effect. PA' s bitterness suppression stems from its ability to adsorb bitter substances and mask TAS2Rs. When bitter compounds enter the oral cavity, they preferentially bind to PA, reducing TAS2R-bitter substance binding and thereby inhibiting bitter taste generation. Nakamura et al. [32] reported that 1% PA reduced the bitterness of 0.1 mol/L quinine hydrochloride by 81.7%, with masking effects accounting for 45.6% of this reduction.

PA is present in barley germ, corn germ, soybeans, and egg yolk, but in scarce quantities. Therefore, PA is typically produced by enzymatic hydrolysis of lecithin using phospholipase D.

4.4 4-(2,2,3-Trimethylcyclopentyl)butanoic Acid (GIV3727)

GIV3727 is a small-molecule bitter taste receptor antagonist that inhibits the reaction between bitter substances in saccharin and acesulfame with TAS2R31. It also inhibits five other TAS2Rs, including the bitter taste-critical TAS2R43. Slack et al. [33] constructed homology models of TAS2R31 and TAS2R43 based on β-adrenergic crystal structures and found that GIV3727' s interaction with TAS2Rs depends on two vicinal residues in the receptors. GIV3727 can mutate the corresponding sites of these residues and enhance receptor selectivity for antagonists, allowing GIV3727 to bind at the binding site and reduce TAS2R mRNA expression, thereby decreasing bitter taste.

Additionally, substances such as cyclodextrins, fructans, and tannic acid can compete with bitter substances for TAS2R binding. By preferentially reacting with the TAS2R membrane surface and modulating TAS2R channels, they inhibit bitter molecule-TAS2R interactions, reducing bitterness without affecting other taste receptors' perception of sour, sweet, salty, and umami tastes.

As a taste receptor, TAS2Rs are expressed not only in tongue taste buds but also in airway smooth muscle, gastrointestinal tract, cerebellum, and testis of animals. At least three bitter signal transduction pathways have been identified, reflecting the diverse chemical structures of bitter substances. However,

since bitterness causes aversion in animals, inhibiting the binding of feed bitter substances to TAS2Rs to improve palatability is crucial. Therefore, in-depth research on bitter receptor blockers holds significant importance for enhancing animal feed intake and improving animal welfare.

References

[1] MEYERHOF W, BATRAM C, KUHN C, et al. The molecular receptive ranges of human TAS2R bitter taste receptors[J]. Chemical Senses, 2010, 35(2): 157–170.
[2] Hu Lingling, Shi Peng. Recent advances in the function and evolution of bitter taste receptor gene family[J]. Chinese Science Bulletin, 2009, 54(17): 2472–2482.
[3] Zhong Yuan, Pan Yang, Hou Wenhai, et al. Molecular cloning and sequence analysis of the black bear bitter taste receptor TAS2R2 gene[J]. Chinese Journal of Wildlife, 2014, 35(4): 407–413.
[4] Wei Chengxiao. Cloning and sequence analysis of the TAS2R1 gene in Landrace pigs[J]. Swine Industry Science, 2015, 32(12): 102–104.
[5] EINSTEIN R, JORDAN H, ZHOU W Y, et al. Alternative splicing of the G protein-coupled receptor superfamily in human airway smooth muscle diversifies the complement of receptors[J]. Proceedings of the National Academy of Sciences of the United States of America, 2008, 105(13): 5230–5235.
[6] SHAH A S, BEN-SHAHAR Y, MONINGER T O, et al. Motile cilia of human airway epithelia are chemosensory[J]. Science, 2009, 325(5944): 1131–1134.
[7] WU S V, CHEN M C, ROZENGURT E. Genomic organization, expression, and function of bitter taste receptors (T2R) in mouse and rat[J]. Physiological Genomics, 2005, 22(2): 139–149.
[8] SINGH N, VRONTAKIS M, PARKINSON F, et al. Functional bitter taste receptors are expressed in brain cells[J]. Biochemical and Biophysical Research Communications, 2011, 406(1): 146–151.
[9] ROZENGURT E. Taste Receptors in the Gastrointestinal tract . Bitter taste receptors and α-gustducin in the mammalian gut[J]. American Physiological Society, 2006, 291(2): G171–G177.
[10] BEHRENS M, MEYERHOF W. Gustatory and extragustatory functions of mammalian taste receptors[J]. Physiology & Behavior, 2011, 105(1): 4–13.
[11] PRONIN A N, TANG H X, CONNOR J, et al. Identification of ligands for two human bitter T2R receptors[J]. Chemical Senses, 2004, 29(7): 583–593.
[12] SHI P, ZHANG J Z, YANG H, et al. Adaptive diversification of bitter taste receptor genes in mammalian evolution[J]. Molecular Biology and Evolution, 2003, 20(5): 805–814.
[13] LI D Y, ZHANG J Z. Diet Shapes the evolution of the vertebrate bitter taste receptor gene repertoire[J]. Molecular Biology and Evolution, 2014, 31(2): 303–309.
[14] VANDENBEUCH A, KINNAMON S C. Why do taste cells generate action potentials? [J]. Journal of Biology, 2009, 8: 42.
[15] MARGOLSKEE R F. Molecular mechanisms of bitter and sweet taste trans-

duction[J]. Journal of Biology Chemistry, 2002, 277(1): 1-4.
[16] HE W, DANILOVA V, ZOU S Y, et al. Partial rescue of taste responses of α-gustducin null mice by transgenic expression of α-transducin[J]. Chemical Senses, 2002, 27(8): 719-727.
[17] SCOTT K. Taste recognition: food for thought[J]. Neuron, 2005, 48(3): 455-464.
[18] KIM K S, EGAN J M, JANG H J. Denatonium induces secretion of glucagon-like peptide-1 through activation of bitter taste receptor pathways[J]. Diabetologia, 2014, 57(10): 2117-2125.
[19] BHAT T K, KANNAN A, SINGH A, et al. Value addition of feed and fodder by alleviating the antinutritional effects of tannins[J]. Agricultural Research, 2013, 2(3): 189-206.
[20] BILIĆ-ŠOBOT D, KUBALE V, ŠKRLEP M, et al. Effect of hydrolysable tannins on intestinal morphology, proliferation and apoptosis in entire male pigs[J]. Archives of Animal Nutrition, 2016, 70(5): 378-388.
[21] SOARES S, KOHL S, THALMANN S, et al. Different phenolic compounds activate distinct human bitter taste receptors[J]. Journal of Agricultural and Food Chemistry, 2013, 61(7): 1525-1533.
[22] MANI V, HOLLIS J, GABLER N K. Bitter compounds decrease gastric emptying and influence intestinal nutrient transport[J]. Animal Industry Report, 2012, 658(57): R2725-R2728.
[23] TAN J, ABROL R, TRZASKOWSKI B, et al. 3D Structure prediction of TAS2R38 bitter receptors bound to agonists phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP)[J]. Journal of Chemical Information and Modeling, 2012, 52(7): 1875-1885.
[24] ROLAND W S, VINCKEN J P, GOUKA R J, et al. Soy isoflavones and other isoflavonoids activate the human bitter taste receptors hTAS2R14 and hTAS2R39[J]. Journal of Agricultural and Food Chemistry, 2011, 59(21): 11764-11771.
[25] Li Lei, Zhou Shengsheng. Research progress on bitter plant compounds and bitter taste modifiers[J]. Food Industry, 2014, 35(6): 213-217.
[26] LEY J P. Masking bitter taste by molecules[J]. Chemosensory Perception, 2008, 1(1): 58-77.
[27] PYDI S P, SOBOTKIEWICZ T, BILLAKANTI R, et al. Amino acid derivatives as bitter taste receptor (T2R) blockers[J]. Journal of Biological Chemistry, 2014, 289(36): 25054-25066.
[28] BYLUND D B, TOEWS M L. Quantitative versus qualitative data: the numerical dimensions of drug action[J]. Biochemical Pharmacology, 2014, 87(1): 25-39.
[29] CHAKRABORTY R, BHULLAR R P, DAKSHINAMURTI S, et al. Inverse agonism of SQ 29,548 and ramatroban on thromboxane A2 receptor[J]. PLoS One, 2014, 9(1): 85937-85960.
[30] Wang Weijiang, Zheng Jianxian. Research and application of natural bitter taste inhibitors[J]. China Condiment, 2006(2): 13-16, 53.
[31] MARGOLSKEE R F, DING M. Inhibitors of the bitter taste response: US, USRE40594[P]. 2008.

[32] NAKAMURA T, TANIGAKE A, MIYANAGA Y, et al. The effect of various substances on the suppression of the bitterness of quinine-human gustatory sensation, binding, and taste sensor studies[J]. Chemical and Pharmaceutical Bulletin, 2002, 50(12): 1589–1593.
[33] SLACK J P, BROCKHOFF A, BATRAM C, et al. Modulation of bitter taste perception by a small molecule hTAS2R antagonist[J]. Current Biology, 2010, 20(12): 1104–1109.

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