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Bitter Taste, Rising New Functions and Significance of Extra-oral Expressions

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Taste is closely related to intake of food. Taste perception is also influenced by type of food ingested, and nutrition and health status. Bitter taste plays an important role in the survival of human and animals to avoid probable toxic and harmful substances. Vertebrate animals recognize bitter taste through type 2 taste receptors (T2Rs). Several T2Rs have been expressed extra-oral such as the gastrointestinal tract, respiratory tract, urogenital tract, brain and immune cells, and parts of their functions are being revealed. This review will discuss physiological roles of T2Rs in relation to innate immunity, secretion and smooth muscle contraction expressed in extra-oral cells and tissues, and we summarize relationships between polymorphisms in T2Rs and general or oral diseases. It is not a coincidence that animals pay much genetic costs for taste and smell during evolution.

Key words: taste, bitter, receptors, oral diseases, chemoreception, polymorphism

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Basic taste and bitter taste

The taste is closely related to the intake of food, and it is also influenced by the type of food ingested, and nutritional and healthy status. The human tastes consist of five qualities which is attractive or aversive to foods. The bitter taste plays an important role in the survival of human and animals to avoid probable toxic and harmful substances. The vertebrate animal senses bitter taste through type 2 taste receptors (T2Rs), a kind of G protein-coupled receptors which is exclusively expressed in the type 2 taste cells among four types of taste bud cells. Approximately 5% of the about 20,000 total genes in human are involved in chemosensory reception, of which more than 30 genes are related in taste transduction. In human, 25 T2Rs were identified in the oral cavity [1]. The vertebrate T2Rs differ in the number of genes in each species. There are 3 types T2R in chicken, 15 types in dog, 12 types in cow, and 35 types in mice [2], which suggest that polymorphism of bitter taste receptor would be evolved.

Several T2Rs are expressed extra-oral such as gastrointestinal tract, respiratory tract, urogenital tract, brain and immune cells through various studies, and parts of their functions are being revealed.

The purpose of this review is to investigate expression patterns, localizations, and/or probable relationships between diseases.

T2Rs signaling pathway

General T2Rs signaling pathway shares signaling molecules sweet and umami tastes such as G protein subunit, phospholipase C (PLCβ2), inositol trisphosphate receptor (IP3R) and transient receptor potential cation channel M5 (TRPM5) [3]. Activated PLCβ2s by bitter taste substances produce IP3s which release Ca^{2+} from the intracellular Ca^{2+} reservoir and resulting Na^{+} influx through the TRPM5 channels. The depolarization of taste cells due to Na^{+} influx, resulting in secretion of neurotransmitter ATP through gap junctions or CALHM1 ion channels [4, 5]. ATP activates the purinergic receptors in type 3 taste bud cells or taste nerves, and the signals from taste bud project to the central nervous system as bitter taste [5].

Bitter taste receptors functions at the cellular level

Extra-oral T2Rs use at least three different mechanisms to play biological roles depending on the locations of expression. The three mechanisms are the same in the general signaling system of T2Rs until the process of activating the receptors and increasing the intracellular Ca^{2+} concentration in taste cells. However, their functions are different from their location found. Three mechanisms can be divided into cell-autonomous regulation, paracrine regulation, and endocrine regulation.

Cell-autonomous regulation of T2Rs is mainly found in the motile cilia of airway epithelial cells of human [6]. This type of cellular response depends on the doses of the bitter substances. It elicited an increase of Ca^{2+} and consequently accelerated ciliary beat frequency [7]. Other cell-autonomous control happens in the airway smooth muscles, which relax airway smooth muscles depending on the bitter substance doses [8]. The βγ subunits of g proteins can block L-type voltage-dependent Ca^{2+} channels and reduce Ca^{2+} influx, resulting in relaxation of the airway smooth muscles [9].

The paracrine regulation of T2Rs was reported in enteroendocrine cell (EEC). Increased Ca^{2+} as a result of activation of T2Rs promotes the secretion of cholecystokinin (CCK). CCK promotes multidrug-resistant protein 1, known as ATP-binding cassette B1 (ABCB1) and acts on CCK1 receptors in the sensory fibers of the vagus nerve, which transmits brain signals that regulate food intake.

It has been found that solitary chemosensory cells (SCCs) of nasal cavity and vomeronasal organ (VNO), or brush cells in the trachea of rodents, secrete acetylcholine in response to bitter taste chemical or bacterial signals. Acetylcholine activates the nicotinic acetylcholine receptors of the sensory nerve fibers, which reduces the respiratory rate, closes VNO, or causes neurogenic inflammation in the nasal cavity [10]. Similar protective reflexes have also been found in urethral brush cells of the bladder [11]. Gut brush cells form a feedforward loop by organizing the type 2 immune system and causing cell hyperplasia to parasite infestation through a general GPCR taste sensory system [12]. The endocrine regulation for T2Rs signal is that when the receptors are activated, the hormone secretes and then acts on the tissue or cells. Intestinal EEC secretes glucagonlike peptide 1 (GLP-1) and stimulates the secretion of insulin from the pancreatic β-cells [13].

Physiological roles of extra-oral T2Rs

Immunity

Many studies on the correlation between innate immune responses and bitter substances have focused on the respiratory system. A variety of T2Rs are expressed in the ciliated epithelial cells of human and rodents. Activation of T2R4, T2R43, and T2R46, expressed in human ciliated epithelial cells, by bitter chemicals increases Ca^{2+} influx and ciliary beat frequency, then accelerates the clearance of microbial-generated products [6]. A T2R38 agonist or microbe-derived quorum-sensing molecule, acyl-homoserine lactones (AHLs), binds to T2R38 in the apical membrane and cilia of sinus epithelium, producing nitrogen oxide, a potent bactericide [14]. When the concentration of quorum-sensing molecules is high enough, the biofilm is formed to protect bacteria from the host immune defense system [14]. The SCC is one of the airway epithelial cells with T2Rs and most taste transduction components and constitutes, and consists of about 1% of the surface of the respiratory system [15]. Finger *et al*. [16] first identified T2Rs-expressing SCCs in rodent nasal cavity. The SCC also has bitter taste signaling components such as α-gustducin, PLCβ2 and TRPM5 [17, 18]. Bitter taste substances or AHL cause the mouse nasal SCCs to secrete acetylcholine, which stimulates neighboring nociceptive trigeminal fibers to stimulate secretion of calcitonin gene-related peptide and substance P, resulting in the initiation of a neurogenic

inflammation response to block bacterial invasion. The reaction inhibits the inhalation of stimulants or microorganisms by linking protective reflexes such as respiratory rate reduction [10, 17]. Harmful bitter taste substances bind with T2Rs expressed in the brush cells of the urethral system activates the urethral sensory nerve fibers and causes detrusor muscle contraction with similar manners [11].

The brush cells within gastrointestinal tract are known to detect parasitic infections through general taste signal transduction and secrete IL-25, which increases the number of innate lymphoid cells and their production of type 2 immune cytokines IL-4 and IL-13. Subsequently, cytokine promotes the hyperplasia of brush cells and goblet cells by promoting intestinal stem cell differentiation. However, it is not known exactly which T2Rs are involved or whether it is caused by another receptor [12]. It was reported that T2R transcripts were expressed in polymorphonuclear neutrophils. Knockdown of T2R43 or T2R31 in neutrophils significantly blocks chemotactic trans-migration induced by saccharine [22]. Other studies have reported that T2R38, which is expressed in human neutrophils, binds to the quorum-sensing molecule AHL-12 and causes neutrophil migration [23]. Phagocytes also express T2R38, which can be activated by AHL-12 [24].

Activation of T2Rs expressed in SCCs transmits Ca^{2+} to the surrounding cells through gap junctions and leads to secretion of antimicrobial peptides and β-defensin, however, it does not affect ciliary beat frequency [20]. In addition, the SCCs express sweet taste receptors, T1R2/3, which act in opposition to T2Rs. Activation of the airway surface with a glucose solution inhibits the secretion of antimicrobial peptides mediated by T2Rs. However, when infected with microorganisms, T1R2/3 is deactivated because bacteria consume glucose, consequently the antimicrobial peptide secretion by T2Rs increases [21]. Taken together, the results suggest that bitter substances may function in the immune system.

Secretion

T2Rs expressed in respiratory epithelial cells mediate the secretion of nitrogen oxides, neurotransmitters and antimicrobial peptides [20]. One of the roles of T2Rs in gastrointestinal epithelial cells is to limit their effects on toxic substances by limiting their consumption or promoting their excretion. EECs present in the epithelial layer of the gastrointestinal tract from the stomach to the rectum responds to food ingested by secreting various digestive hormones such as CCK, GLP-1, glucose-dependent insulinotropic peptide, peptide YY, somatostatin, ghrelin and serotonin [25]. Secretion of these hormones is mainly stimulated by luminal contents via GPCRs such as T2Rs [26]. Denatonium, a bitter substance, stimulates CCK secretion in STC-1 of the EEC line of mice [27]. Bitter taste substances or herbal extracts induce GLP-1 secretion in human EEC line NCI-H716 [28]. *In vivo* experiments show that the EECs can secrete hormones to regulate plasma glucose or toxic substances intake. Direct administration of bitter substances in the stomach leads to a rise in the plasma ghrelin levels and then increases short-term food intake. As a result, it reduces long-term food intake and delays in gastric emptying [29]. A gavage of denatonium followed by glucose or oral administration of herbal extracts to db/db mice induces GLP-1 and subsequent insulin secretion, thereby reducing blood glucose levels [13, 27]. Even though α-gustducin and TRPM5 were expressed in EECs, the co-localization of them and EEC markers did not confirm [30]. These results suggest that the glucose drop is caused exclusively by EECs.

The ligand of murine t2r108, 6-n-propyl-2-thiouracil (6-PTU), causes secretion of anions in the large intestine of the rat [31]. This action is considered to be a reflex action that can excrete harmful stimulants. T2Rs expressed in mouse thyrocytes negatively regulate thyroid-stimulating hormone-dependent iodide efflux, thus reducing the secretion of thyroid hormones, which can act as a protective reflection of ingestion of toxic substances [32]. The secretion of murine salivary glands is probably related to taste. The expression of T2Rs in various exocrine glands in rat and mouse were reported [33, 34]. T2Rs expressed in von Ebner glands and submandibular gland cells of rats responded to both quinine and PTU in a dose-dependent manner [33]. It was also reported that mouse *tas2r108* was the most expressed in exocrine glands such as salivary glands, lacrimal glands, paracrine glands [34]. Expression levels of *tas2r108* in the submandibular gland were higher in acinar cells than in ductal cells. Thus, *tas2r108* expressed in the submandibular gland may influence in both saliva secretion and modification of saliva composition, however, its contribution is more on saliva secretion [35]. These studies suggest that *tas2r108* may detect harmful substances that enter the body and secrete saliva, diluting harmful substances.

Contraction of smooth/cardiac muscles.

Many researchers are paying attention to T2Rs that expresses in smooth muscle. T2Rs agonists relax pre-contracted airway

smooth muscle and reduce airway resistance in mice [8]. The bitter taste substance directly inhibited IP3R-associated Ca^{2+} oscillations to relax the airway [37]. Tazzeo *et al*. [38] suggested that a bitter substance, caffeine, acts on the downstream of the myosin light chain kinase to object to the contractile apparatus, causing the airway smooth muscle relaxation. The bitter taste substances would be used as a bronchodilator. Various bitter substances have been shown to relax the smooth muscle of pre-contractile airways of human, mice, and guinea pigs [37, 39, 40]. The advantage of using bitter taste materials as a bronchodilator is that it can cause pre-contracted relaxation and most bitter taste receptors have a broad spectrum [40]. It should be assessed the effectiveness of each substance when using a bitter taste substance as a bronchodilator.

Zhai *et al*. [41] reported that human and mouse detrusor smooth muscle express T2Rs, and the agonist of these receptors relaxed the pre-contraction detrusor muscle. It was also reported that overactive bladder symptoms in mouse were suppressed by oral administration of chloroquine, a bitter substance. Therefore, T2R would be a therapeutic target for this disease. Several studies have demonstrated that bitter substances control smooth muscle contraction in blood vessels. Upadhyaya *et al*. [42] reported that dextromethorphan leads to vasoconstriction through T2R1-associated Ca^{2+} response in human pulmonary artery smooth muscle. According to reports, the increase in Ca^{2+} associated with canonical T2Rs signaling system directly activates myosin light chain kinase [42]. Applying bitter taste substances at low concentrations (eg, denatonium <100 μM) causes muscle contraction, and high concentrations (eg, denatonium> 500 μM) result in muscle relaxation in mouse and human gastrointestinal smooth muscle cells. During an oral nutrient challenge test on healthy subjects, denatonium elicited an impaired fundic relaxation in response to nutrient infusion and a decreased nutrient volume tolerance and increased satiation [43].

Five types of T2Rs and downstream signaling elements were expressed in cardiac myocytes [44]. Sodium thiocyanate, t2r108 agonist in mice, reduced left ventricular and systolic pressures by 30-40%, as well as increased aortic pressure [45]. These actions disappeared when Gi and Gβγ inhibited.

Male reproduction and micturition

T1Rs and taste transducers cascade components such as α-gustducin, Gγ13 and PLCβ2 were identified in different stages of spermatogenesis [46]. Bitter taste substances lead to increased calcium influx into sperm cells, and each sperm cell has different activation for ligands [47]. The decrease in *tas2r105* was made sperms smaller and it could result in male infertility [46]. It is believed that T2Rs play an important role in sperm survival by detecting harmful substances during fertilization.

Seven T2Rs and α-gustducin were expressed in mouse kidney [48]. Knockout of *tas2r105*-positive cells in mouse increased the size of glomerulus and renal tubules and decreased the density of glomerulus [48]. These results suggest that *tas2r105* plays an important role in maintaining the homeostasis of body fluids and electrolytes [48].

T2Rs may regulate various functions in relation to reproductive and urination, but it is inadequate to study such as respiratory or digestive systems.

Polymorphisms of T2Rs

T2Rs polymorphisms are an important research object that can clarify the taste preference and the pathophysiology of extra-oral T2Rs. Among T2Rs, T2R38 polymorphism is the most studied. The T2R38 protein can be divided into two groups according to positions 49, 262 and 296 of amino acid residues. The two mutated proteins are divided into PAV, which is a genotype including the functional mutation proteins proline, alanine and valine, and the AVI genotype, which is a genotype including alanine, valine and isoleucine. The binding of these two mutant proteins is expressed in three genotypes (PVA/PVA, AVI/AVI and PVA/AVI). The relationship between T2R38 polymorphism and respiratory disease has been reported. The T2R38 PVA/PVA phenotype showed a much lower infection rate of gram-positive bacteria than those with two other genotypes [49]. In addition, 90% or more of patients with the non-functional T2R38 genotype showed chronic sinusitis [50]. Those with the AVI/AVI genotype showed more severe sinusitis [50]. T2R38 polymorphism has also been associated with cancer and dental caries. Carrai *et al*. [51] reported that the nonfunctional group increased the risk for rectal cancer compared to the functional group. T2R38 polymorphism is also reported to affect oral innate immunity. The transcription level of T2R38 in periodontal epithelial cells was increased 4.3-fold in PAV/PAV genotype and 1.2-fold in AVI/AVI genotype for the cariogenic bacteria *Streptococcus mutans* [51]. IL-1α secretion of the PAV/PAV genotype was the highest among

the three types of T2R38 proteins. Stimulation with periodontal pathogen *Porphyromonas gingivalis* increased the AVI/AVI T2R38 transcription levels by 4.4-fold [52]. These studies suggest that the risk for periodontal immunity and dental caries is more important for T2R38 than for eating habits [53]. Whether the results of the study were only revealed by the T2R38 gene polymorphism or similar results for other taste receptors genes should also be investigated.

T2Rs and diseases.

Many studies have reported that T2Rs mutations can cause disease in extra-oral tissues and emphasize the importance of T2Rs. T2Rs expressed in airway smooth muscle relaxes muscles in response to bitter substances to reduce airway resistance to asthma. Robinett *et al*. [54] observed that T2R10, 14 and 31 agonists relax airways in both healthy and asthmatic patients. In severe asthma patients, T2Rs are up-regulated in leukocytes and these agonists can inhibit proinflammatory cytokines and eicosanoid secreted by leukocytes [55]. T2Rs agonists can control anti-inflammation and act directly on immune cells. These results demonstrate that T2Rs can be an important target for asthma treatment.

T2Rs pathophysiological roles have focused on the respiratory tract, but more recently, many studies have been conducted on the roles of other tissues. Low-fat food or sterol depleted culture induces increased expression of most T2Rs in the intestine or STC-1 cell line of mice, stimulating the secretion of GLP-1 and CCK [56]. The number of T2R38 immunoreactive cells expressing in the human colon mucosa is significantly increased in overweight or obese subjects and is closely related to body mass index [57]. Expression of t2r126, 135 and 143 in the heart of mice increased two to three fold at fasting [44]. Upon subcutaneous injection of nitroglycerin, t2r119 rapidly increased in the heart and aorta of mice [58]. T2R5 and T2R50 decreased in the brain of patients with Parkinson's disease, whereas T2R10 and T2R13 increased [59]. Interestingly, T2R4, 5, 14, and 50 were decreased in the entire dorsolateral prefrontal cortex of schizophrenia patients [64]. Since the heart is not directly exposed to the external environment and the brain is separated by the blood-brain barrier, there is a possibility that an endogenous ligand exists in the human body which causes the response of T2Rs in the heart and brain.

T2Rs have also been found in tumors or cancer cells. It has been suggested that T2R4 expression reduced in breast cancer patients, thereby decreasing the apoptosis caused by bitter substances in breast cancer cells [60]. T2R38 is known to be expressed in tumor cells and tumor-derived cell lines of pancreatic cancer patients [65]. The T2R38 specific ligand phenylthiourea, or a natural ligand AHL-12, activates mitogen-

Table 1. T2R-associated disorders and diseases in human

type	tissue/organs/system	effects	references
T2R38	upper respiratory system	genotype is correlated with susceptibility, severity, and prognosis of chronic rhinosinusitis	14, 20, 50
	colorectal cancer	nonfunctional group has an increased risk of colorectal cancer	51
	gingiva	genotype is associated with gingival innate immunity and the risk of dental caries	53
	colonic mucosa	increased number of immunoreactive cells in overweight and obese subjects	57
T2R19	blood glucose	haplotype is associated with altered glucose and insulin homeostasis	61
T2R50	heart	SNPs have a strong association with cardiovascular disease	62
T2R42	thyroid	thyroid-expressed SNP (type L196F) is associated with differences in circulating levels of thyroid hormones	32
T2R16	longevity	an upstream position polymorphism is associated with longevity	63
T2R4	breast cancer	T2R4 is down-regulated in breast cancer cells.	60
T2Rs	leukocytes	10 T2Rs are up-regulated in leukocytes in severe asthma patients	55
	Parkinson's disease patients' brains	T2R5 and T2R50 are decreased, whereas T2R10 and T2R13 are augmented at both premotor and parkinsonian stages in the frontal cortex area	59
	schizophrenia patients' brains	T2R4, T2R5, T2R14, and T2R50 are down-regulated in the dorsolateral prefrontal cortex	64

Table 2. t2r-associated disorders and diseases in mouse

type	tissue/organs/system	effects	reference
t2r105	testes	depletion of T2R105 results in smaller testes and leads to male infertility	46
	glomerulus and renal tubule	ablation of T2R105-positive cells causes an increase in the size of the glomerulus and renal tubule and a lower cell density in the glomerulus	48
t2r126, t2r135, t2r143	heart	starvation increases the expression of these T2Rs by two- to threefold	44

activated protein kinases p38 and ERK1/2 and increases NFATc1 in a G protein-dependent manner. T2R38-positive tumors were not related to the clinical and pathologic parameters, but the T2R38 ligand increased the expression of ABCB1 and seems to be associated with pancreatic cancer resistance and T2R38 [65].

Perspectives

T2Rs expressed in extra-oral cells or tissues is continuously being discovered and its function is being revealed. Studies on T2Rs have been carried out on the pathophysiology of the respiratory tract. Based on the action of a bitter taste substance on T2Rs, a new approach to the treatment of asthma is presented by developing a bronchodilator. Recently, the expression of T2Rs in cancer cells was reported. The results of previous studies show that there is a need to study with interest the pharmacogenetics related to T2Rs and these polymorphisms.

There is a continuing interest in research on the relationship between taste receptors and oral diseases. Studies have also reported that the T2R38 polymorphism is associated with dental caries and periodontal disease. It has been also suggested that taste disorders would associate with burning mouth syndrome.

The taste is an important reflex stimulus for saliva formation, and the saliva in the mouth is an essential factor for the taste. We reported the expression of T2Rs in the submandibular glands of mice and rat. Although the exact physiological role has not yet been clarified, T2Rs may play a role in protecting the organism by causing secretion of saliva. The expression levels of T2Rs in salivary glands of mice were also different. The expression level of *tas2r108* among 35 T2Rs was remarkably high, and it can be assumed that 35 T2Rs may not play the same physiological role [66]. Therefore, it is valuable to study elucidating the reason of uneven expression of T2Rs in mammals.

Many studies show that oral diseases and taste are related to each other. In order to predict, diagnose and treat oral diseases, it is necessary for oral professionals to pay attention to taste and conduct in depth studies.

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