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Functional characterization of the heterodimeric sweet taste receptor T1R2 and T1R3 from a New World monkey species (squirrel monkey) and its response to sweet-tasting proteins

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ABSTRACT

The family C G protein-coupled receptor (GPCR) T1R2 and T1R3 heterodimer functions as a broadly acting sweet taste receptor. Perception of sweet taste is a species-dependent physiological process. It has been widely reported that New World monkeys and rodents are not able to perceive some of the artificial sweeteners and sweet-tasting proteins that can be perceived by humans, apes, and Old World monkeys. Until now, only the sweet receptors of humans, mice and rats have been functionally characterized. Here we report characterization of the sweet taste receptor (T1R2/T1R3) from a species of New World primate, squirrel monkey. Our results show that the heterodimeric receptor of squirrel monkey does not respond to artificial sweeteners aspartame, neotame, cyclamate, saccharin and sweet-tasting protein monellin, but surprisingly, it does respond to thaumatin at high concentrations (>18 μM). This is the first report demonstrating that species of New World monkey can perceive some specific sweet-tasting proteins. Furthermore, the sweet receptor of squirrel monkey responses to the such sweeteners cannot be inhibited by the sweet inhibitor lactisole. We compared the response differences of the squirrel monkey and human receptors and found that the residues in T1R2 determine species-dependent sweet taste toward saccharin, while the residues in either T1R2 or T1R3 are responsible for the sweet taste difference between humans and squirrel monkeys toward monellin. Molecular models indicated that electrostatic properties of the receptors probably mediate the species-dependent response to sweet-tasting proteins.

1. Introduction

The taste qualities for humans and other mammals can be categorized as sweet, bitter, sour, salty and umami [1]. The heterodimer of T1R2 and T1R3 was identified as a broadly acting sweet taste receptor. In addition to sucrose, the human T1R2/T1R3 receptor responds to all other sweet-taste stimuli tested: natural sugars, sweet amino acids, sweet-tasting proteins, and artificial sweeteners [2–5]. Previous molecular biology experiments using sweet taste receptor chimeras and mutants and molecular modeling studies showed that there are at least five potential binding sites of the sweeteners in the heterodimeric receptor [6–16]. Receptor activity induced by the artificial sweeteners aspartame and neotame implicate residues in the Venus Flytrap Module (VFTM) of human T1R2 [6,10,15,16], while natural sugars bind to the VFTMs of both T1R2 and T1R3 [12]. In contrast, the sweeteners cyclamate and neohesperidine dihydrochalcone (NHDC) [8,13], and the sweet-taste inhibitor lactisole acts on the Transmembrane Domain (TMD) of human T1R3 [9,14], and the sweetener SWT819 acts on the TMD of human T1R2 [17]. Furthermore, receptor activity toward the sweet protein brazzein depends on the cysteine rich domain (CRD) of human T1R3 [7].

Many physiological and molecular biological studies have shown that some sweeteners, such as the small molecule sweeteners aspartame, neotame and cyclamate, and sweet-tasting proteins can be perceived by humans, apes and Old World monkeys, but not by New World monkeys and rodents [18–21]. Thus, an intriguing question is what the molecular basis of species-dependent sweet taste toward these sweeteners as well as the activation mechanism is. In this regard, it is necessary to investigate the properties of sweet taste receptors from different species. Previously, only the sweet receptors of humans, mice and rats had been well studied [6–9,11]. However, the structure and function of sweet taste receptors from New World monkeys, which share nearly 90% sequence identity with the receptors of humans, have not been well studied.

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Recently, we demonstrated the molecular basis of species -dependence of sweet taste receptor toward artificial sweeteners aspartame and neotame by using human/squirrel monkey chimera receptors, mutagenesis and molecular modeling [15]. In this study, we further characterized the newly cloned sweet taste receptors from squirrel monkeys (*Saimiri sciureus*), which belong to the genus *Saimiri* of New World monkeys. We used heterologous expression and calcium mobilization assay to assess the function of the heteromeric receptors (T1R2 and T1R3) from squirrel monkeys (named smT1R2 and smT1R3, respectively). By comparing the functional properties of the sweet taste receptors with those of humans and mice and by using human/squirrel monkey chimeric T1R2/ T1R3, we demonstrated that the residues in T1R2 determine species-dependent sweet taste toward saccharin, while the residues in either T1R2 or T1R3 mediate the sweet taste difference between humans and squirrel monkeys toward monellin. Molecular models indicated that electrostatic properties of the receptors probably mediate the species-dependent response to sweet-tasting proteins.

2. Materials and methods

2.1. Materials

Aspartame, saccharin, cyclamate, sucrose, D-tryptophan, NHDC, lactisole, monellin, and thaumatin were obtained from Sigma–Aldrich. Sucralose was obtained from Splendex. Neotame was obtained from American Health Foods & Ingredients. Stevioside was obtained from Nusci Institute & Corp. Unless noted, the concentration of the compounds used were: aspartame (2.5 mM), neotame (0.25 mM), saccharin (1 mM), cyclamate (10 mM), sucrose (150 mM), sucralose (1 mM), D-tryptophan (5 mM), stevioside (1 mM), neohesperidin dihydrochalcone (0.25 mM), monellin (37 μM), thaumatin (18 μM), and lactisole (1.25 mM) based on the previous studies [7,22].

2.2. Constructs and calcium mobilization functional assay

The coding nucleotide acid sequence and deduced amino acid sequence of squirrel monkey T1R2 and T1R3 were as described previously [23,24]. To generate the smT1R2 expression construct, BamHI and NotI sites were introduced at the 5′ and 3′ ends of the full-length coding sequence, respectively, double digested, and then ligated into the expression vector pcDNA3.1. To obtain the smT1R3 construct, the full protein-coding region of smT1R3 was synthesized from the GenScript Biology CRO. An EcoRI site was then introduced at the 5′ end before the start codon, and a NotI site was introduced at the 3′ end after the stop codon. The smT1R3 gene was double restriction digested with EcoRI and NotI and then ligated into the expression vector pcDNA3.1. Human T1R2 (hT1R2) and T1R3 (hT1R3) expression constructs were generated in pcDNA3.1 vectors. The Gα16-gust44 clone was as described previously [25]. All the constructs were confirmed by DNA sequencing. Calcium mobilization functional assay was as described previously [15].

2.3. Molecular modeling

We constructed homology models of the VFTM-CRD of T1Rs from the human and squirrel monkey based on metabotropic glutamate receptor (mGluR) crystal structure templates using the MODELLER program [26]. The mGluR is the only family C GPCR with crystal structures of the VFTM-CRD available, which shares ~26% sequence identity with the VFTM-CRD of human T1Rs [27]. The sequence alignment between the template and the models was generated by the ClustalW program. We generated 40 homology models for each species based on the glutamate/gadolinium bound form (closed–closed/A, PDB: 1ISR) of the mGluRI-VFTM [28], and glutamate bound form (closed-closed/R, PDB: 2E4U) of the mGluRII-VFTM-CRD crystal structures [29]. The models were evaluated by the Verify 3D server to identify regions of improper folding. After further manual modifications (merging of fragments with best scoring models and minimizing energy by using SYBYL graphic software package (Tripos Inc.)), the models with the best Verify 3D scores were selected. The stereochemical quality of the models was evaluated by PROCHECK program [30], which showed reasonable ϕ and ψ distributions in the Ramachandran plot. All these assessments indicated that the model structures are reasonable.

3. Results

3.1. Sequence analysis of the squirrel monkey sweet taste receptors

It is well known that the sweet taste receptor is a heterodimer of T1R2 and T1R3 (Fig. 1) [2]. The squirrel monkey (*S. sciureus*) T1R2 gene consists of a 2520 bp coding sequence and encodes an 839 amino acid protein (Genbank accession no: A3QP08), while the T1R3 gene consists of 2559 bp and encodes an 852 amino acid protein (ABD14701). Although sequence identity between these two monomers is low (30%), smT1R2 has high overall identity (blastp, NCBI) with the sweet taste receptors from primates *Callithrix pygmaea* (A3QP09, 93%), *Papio hamadryas* (A3QP07, 89%), *Macaca mulatta* (A3QP01, 89%) and *Pongo pygmaeus*

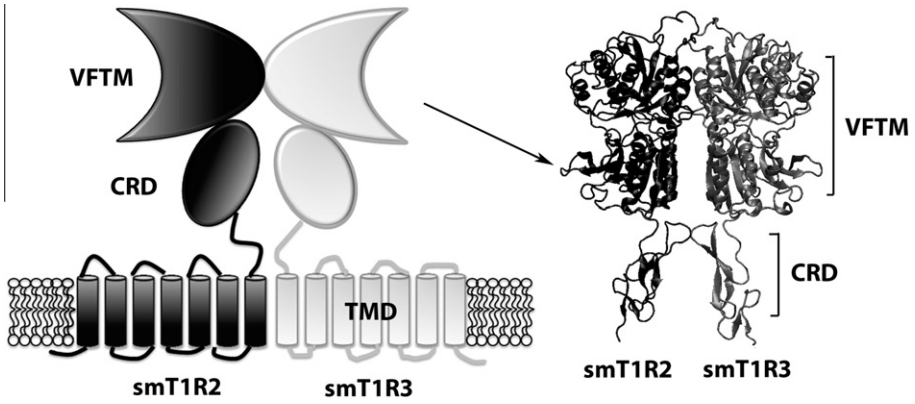

Fig. 1. Schematic representation of the heterodimer of smT1R2 and smT1R3 receptor. The VFTM-CRD of smT1R2/smT1R3 homology model is represented as newcartoon.