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Molecular Mechanisms of Sweet Receptor Function

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Introduction

The T1R taste receptors, like other type 3 G-protein-coupled receptors (GPCRs), have a large amino terminal extracellular domain. Type 3 GPCRs typically function as dimers, but each monomer can independently bind ligand. Based on studies with the metabotropic glutamate receptors (mGluRs) the site for ligand binding in type 3 GPCRs is thought to be in a shell-like cleft formed by two lobes within the extracellular domain. Occupation of the binding cleft and binding to both of the lobes allows the 'shells' to close and stabilizes the active conformation of the receptor (Kunishima *et al.*, 2000; Jingami *et al.*, 2003).

T1R2 + T1R3 monomers form a heterodimeric receptor that is responsive to sweet tasting molecules (unpublished results; Nelson *et al.*, 2001). Small molecule sweeteners can occupy the receptor's extracellular cleft, however, protein sweeteners (e.g. brazzein, monellin and thaumatin) are much too large to fit within this cavity. We hypothesized that these sweet proteins extend 'fingers' into the cleft to occupy the small-molecule binding site.

Using the crystal structure of mGluR1 (Kunishima *et al.*, 2000) to model the extracellular domains of human T1R2 (hT1R2) + hT1R3, we attempted to dock brazzein into the closed cleft of hT1R2. We then mutated residues within the T1R2 to see if they disrupted the response of the expressed receptor to sweeteners.

Results and discussion

The 'sweet finger' model

Using physical and electrostatic constraints we modeled binding of brazzein onto the amino terminal domain (ATD) of hT1R2 such that it would be likely to stabilize the closed conformation by binding to both lobes 1 and 2. We reasoned that because the binding cleft was fairly narrow in the closed position and negatively charged we would need to position brazzein so that a positively charged loop would fit into the cleft. We used electrostatic maps of brazzein (taking into account the effects of mutations on the electrostatic potential; Assadi-Porter *et al.*, 2000) and of hT1R2 to position brazzein (see Figure 1). The model predicts that residues of hT1R2 (E61 and E63 in lobe 1; D278 and D307 in lobe 2) at the mouth of the cleft would interact with brazzein R43 and K42 and be necessary for the receptor's response to brazzein. To test this model we mutated two of these residues to neutral or positively charged residues then tested the mutated receptor's responses to brazzein (see Table 1). Most of these mutations had no effect on receptor responses to brazzein, arguing against this model.

Single mutations in hT1R2 alter ligand-induced activity

We also examined the effects of mutations in hT1R2's ATD on responsiveness to other sweet ligands (D-tryptophan, brazzein, aspartame, sucrose and monellin). Residues E63 and D307 of hT1R2 were predicted by the 'sweet finger' model to interact with brazzein's R43 loop. D307 is also of interest because it corresponds to a known ligand-binding residue in mGluR1. Thus, D307 is predicted to interact with the small molecule sweeteners as well as brazzein. The results of these studies are summarized in Table 1.

The E63A mutation had no effect on responses to any of the ligands tested. The E63K substitution had no effect on responses to brazzein and sucrose, and little if any effect on responses to monellin. This mutant showed reduced activity for D-Tryp and aspartame. If a negative charge is important at position 63 it may be that the negative charge at E61 suffices for some ligands. It is also possible that the presence of E61 somewhat lessened the impact of a positive charge at position 63.

Mutations of D307 (see Figure 1) had large effects on small molecule sweetener activity. The D307A mutation lessened the response to D-Tryp and aspartame. The D307N substitution abolished the responses to D-Tryp, aspartame and sucrose, but had no effect on responses to brazzein or monellin. The D307K substituted receptor marginally responded to brazzein, but did not respond to the other sweeteners at all.

Although these studies are at an early stage they do not support the sweet finger model (Figure 1). That mutations at D307 altered the response to sweet ligands in a selective manner suggests that our model of T1Rs based on alignment with the solved structure of mGluR1 will be productive for investigating sweet receptor-ligand interactions.

Methods

Homology modeling

Based on the crystal structure of the mGluR1 ATD (PDB: 1EWV) we used the MODELLER program (Sanchez and Sali, 1997) to generate a homology model of the heterodimer of human T1R2 and T1R3 ATDs. The sequence alignment between the sweet receptor

Table 1 Responses to sweeteners of mutant sweet receptors. Mutations are at positions E63 or D307 in the ATD of hT1R2. Mutants were paired with hT1R3 and responses were compared to wildtype hT1R2 + hT1R3 receptors

	D-Tryp	Brazzein	Aspartame	Sucrose	Monellin
Wildtype	+++	+++++	+++	+	+++++
hT1R2E63A	+++	+++++	+++	+	++++
hT1R2E63K	++	+++++	++	+	++++
hT1R2D307A	+	+++++	+	+	+++++
hT1R2D307K	–	+	–	–	–
hT1R2D307N	–	+++++	–	–	++++

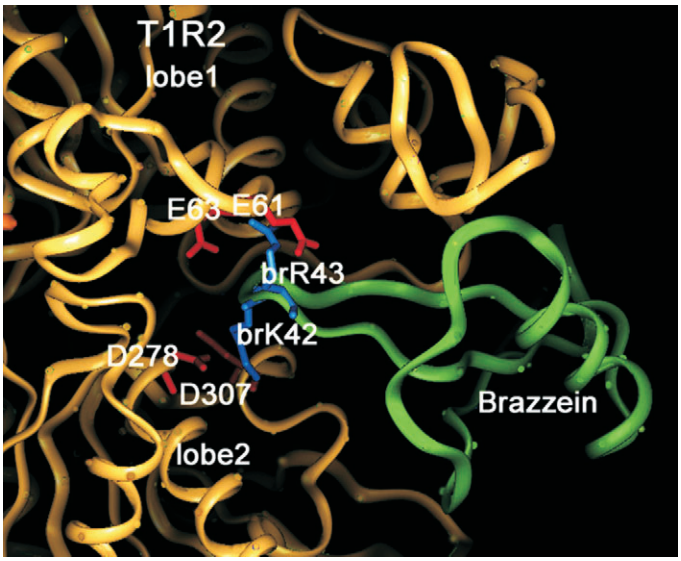

Figure 1 A model of hT1R2 (tan and left) in the closed state looking in to the cleft from the dimerization interface. Lobe 1 is at the top and lobe 2 at the bottom. Brazzein (green and right) is shown with the loop containing Lys42 and Arg43 inserted into the mouth of the hT1R2 cleft. By positioning brazzein thus, the positively charged loop is complementary to the two glutamic acids (Glu61, 63) in lobe 1 and aspartic acids (Asp278, 307) in lobe 2.

and mGluR1 was generated by the ClustalW (http://www.ebi.ac.uk/clustalw/) program (Pearson and Lipman, 1988; Pearson, 1990). The initial homology model was refined using computational simulations (Kolinski and Skolnick, 1998; Ortiz *et al.*, 1999; Skolnick *et al.*, 2000) with the CHARMM program (Brooks *et al.*, 1983; MacKerell *et al.*, 1998). Special consideration was given to the two loop regions of both T1R monomers with low homology to the mGluR1 template. During the refinement using the recently developed MD-PHS approach (Beglov and Roux, 1995; Rosenhouse-Dantsker and Osman, 2000) the two loop regions of both monomers were free to move and the alpha carbon atoms of the remaining parts of the model were restricted by harmonic restraint force to avoid possible distortion.

Construction of T1R mutants, heterologous expression and functional assays

Mutants of hT1R2 were made by site-directed mutagenesis. HEK 293 cells were cultured in 6-well plates at 8×10^5 cells/well. After 24 h in the incubator (37°C, 5% CO_2), the cells were transfected with plasmid DNAs (0.3 μg of each T1R receptor, 0.5 μg of Gα16-i3 and 0.1 μg marker pDsRed2) using Mirus TransIt-293 (Mirus, Madison, WI). At 24 h after transfection the cells were plated onto poly-L-lysine coated 96-well plates. Low-glucose DMEM supplemented with GlutaMAX and 10% dialyzed FBS (Gibco BRL) was used. After an additional 24 h in 96-well plates, the cells were loaded with the calcium dye fluo-4 acetoxymethyl ester (Molecular Probes), washed three times with DPBS, then stimulated by taste compounds over a range of concentrations. Calcium mobilization was monitored on an Olympus fluoview confocal microscope or on a Flex station plate reader. For data analysis, the increase of the fluorescence intensity of an average imaging field was measured.

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